Investigations into Some of the Factors Affecting Prostaglandin Production by Rat and Human Blood Vessels

by

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This thesis was composed by myself and has not been presented in any previous application for a degree. Any help given by other persons has been acknowledged.

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ABSTRACT

The experiments described in this thesis concern the study, in rat and human blood vessels, of the production, and basal and stimulated release of prostaglandins (PGs). The PGs have potent effects on vascular smooth muscle and may be involved in the control of blood flow and blood pressure.

The profile of PG production in homogenates from blood vessels of male and female rats has been characterised. $PGF_{2\alpha}$ and PGE_2 were found to be produced in substantial amounts in addition to the PG more usually measured, PGI_2. There was a difference in the profile of PG production between male and female rats, with the aorta from female rats producing significantly more $PGF_{2\alpha}$ than the aorta from male rats. Also, the basal output of 6-keto-$PGF_{1\alpha}$ was greater from the perfused aorta of male than female rats. However, no difference was observed in PG production by the aorta and vena cava of female rats between Day 1 (oestrus) and Day 4 (prooestrus) of the oestrous cycle. Also, short-term ovariectomy and 2 days of oestrogen and progesterone treatment did not affect PG production by the aorta and vena cava of treated compared to control female rats.

PGs have been implicated in the control of vascular tone, therefore PG production by homogenates of aorta and vena cava from male and female New Zealand genetically hypertensive (GH) rats was investigated. 6-keto-$PGF_{1\alpha}$ and

PGE_2 production by either blood vessel did not differ in normotensive compared to GH male rats. However, $PGF_{2\alpha}$ production was significantly greater by the aorta of male GH rats than of male normotensives. The smooth muscle and endothelial cell layers of GH male rats were found to produce significantly more $PGF_{2\alpha}$ than that of male normotensives. The smooth muscle layer from male GH rats also produced less 6-keto-$PGF_{1\alpha}$ than controls. The isolated, perfused aorta of male GH rats released significantly greater amounts of $PGF_{2\alpha}$, but not of 6-keto-$PGF_{1\alpha}$ or PGE_2 than the aorta from normotensives. The smooth muscle and endothelial cell layers of aorta from female GH rats produced significantly less 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ than those from normotensives. Conversely, the release of all 3 PGs was greater from the isolated, perfused aorta of GH females compared to normotensives. Thus, male GH rats have an increased capacity for the synthesis of the vasoconstrictor PG, $PGF_{2\alpha}$ and show an increased release of $PGF_{2\alpha}$ from the perfused aorta. Female GH rats however, have an increased capacity for the synthesis of PGE_2 and show an increase in the release of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 from the perfused aorta.

PGs may modulate the actions of the pressor hormones noradrenaline (NA) and angiotensin II (AII), therefore the release of PGs after stimulation with NA and AII was determined in the isolated, perfused mesenteric arterial bed from male and female, normotensive and GH rats. In male rats, NA caused a significant increase in

6-keto-$PGF_{1\alpha}$ release in GH and control rats. AII caused a significant increase in 6-keto-$PGF_{1\alpha}$ release in normotensive but not in GH rats. There was a greater pressor response to both NA and AII in the mesenteric bed from GH rats suggesting that PGI_2 alone is not attenuating the response to NA. The absence of any increase in PGI_2 release after AII may reflect a deficiency in this proposed aspect of the control of vascular tone in the GH male rat. In female normotensive and GH rats only the higher dose of NA and AII caused a significant increase in PGI_2 release, with no difference observed in the pressor response between the two groups.

Blood pressure increases with age, consequently blood vessel PG production was measured in aged male and female rats, established to have higher blood pressures than similar young rats. The amounts of 6-keto-$PGF_{1\alpha}$ produced by homogenates of smooth muscle were considerably greater in old, compared to young male and female rats. However, the basal output of 6-keto-$PGF_{1\alpha}$ from the perfused aorta did not change significantly with age, but did tend to be greater in old male and female rats. The production of $PGF_{2\alpha}$ by homogenates, and $PGF_{2\alpha}$ output from perfused aorta was significantly greater in old compared to young rats. The responses to NA and AII in the mesenteric bed did not differ markedly between old and young rats.

In the human study, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 production by homogenates of aorta and saphenous vein were largely unaffected by the age or sex of the

subjects. 6-Keto-$PGF_{1\alpha}$ production by homogenates of saphenous vein was lower in male, but not female, smokers and ex-smokers, although this was significant only for ex-smokers. However, the basal outputs of PGs, particularly 6-keto-$PGF_{1\alpha}$ from the saphenous vein were not affected markedly by age, sex or smoking habits.

Table of Contents

List of Abbreviations

AI	angiotensin I
AII	angiotensin II
AA	arachidonic acid
Ca2+	calcium antagonist
CO	cyclooxygenase
DARS	donkey anti-rabbit serum
GH	New Zealand genetically hypertensive rat
10.00 h	10:00 hours
15-PGHD	15-prostaglandin-hydroxydehydrogenase
NRS	normal rabbit serum
OVX	ovariectomised
Me, TMS	methyl trimethylsilyl ether
Me, BuO, TMS	methyl trimethylsilyl butoxime
NA	noradrenaline
NSAID	non-steroidal anti-inflammatory drug
9-PGE2-KR	9-prostaglandin E2-ketoreductase
PG	prostaglandin
RIA	radioimmunoassay
s.c.	subcutaneous
s.e.m.	standard error of the mean
SHR	spontaneously hypertensive rat

List of Figures

List of Tables

SECTION 1.0

1.1 An introduction to prostaglandin biochemistry

1.1.1 Historical

In 1966, the first prostaglandin (PG) symposium was held in Stockholm. There were 76 participants and 35 papers were read over 2 days. In his opening address, von Euler said "..... we have indeed in prostaglandin a unique hormone..... its scope of action is still wider, however, and it may well be that the prostaglandins represent a group of compounds which are involved in a variety of actions ranging from effects on the central nervous system to intricate metabolic actions, thus justifying their very special chemical configuration". This prophetic statement was easily fulfilled within 10 years, culminating in 1982 with the presentation of the Nobel Prize for Medicine to two of the founding fathers of the PG field, Bengt Samuelsson and Sune Bergstrom, and one of its most respected workers, John Vane. The predicted scope of action and impact of arachidonic acid metabolites on all areas of biology is evident from a consideration of the proceedings of the sixth international prostaglandin conference held in Florence in 1986.

1.1.2 Prostaglandins and their origin

PGs are a group of structurally related compounds implicated in the majority of vital physiological processes such as blood-pressure regulation, haemostasis, inflammation, immune regulation and reproduction. They are produced by almost every cell in the body, with the exception of the red blood cell, and because PGE_2 and $PGF_{2\alpha}$ are metabolised during one passage through the lungs they most closely resemble autocoids, or local hormones. PGI_2 and PGD_2, however, are not metabolised to any great extent by the lung and therefore are potentially able to act as circulating hormones (Armstrong, Lattimer, Moncada and Vane, 1978). Structurally, PGs are derivatives of the hypothetical compound prostanoic acid, which is characterised by 20 carbon (C) atoms, arranged in a cyclopentane ring with two side chains, one of which is terminated by a carboxyl group the other by a methyl group. Thus PGs are 20-C cyclic fatty acids and depending on the structure of the cyclopentane ring are divided into families indicated by the letters A-I. Each family consists of a 1-, 2- and 3-series depending on the number of double bonds in the side chains. PGI_1 cannot be produced biosynthetically and consequently must lack a physiological role.

Before any further discussion of PGs and their many actions in the body is possible, it is neccessary to consider the ultimate origins and biosynthesis of these compounds. Dietary fats consist mainly of fatty acids esterified to glycerol. For a long time fats have been considered solely as a source of energy but this way of

thinking has changed completely since the structural and metabolic functions of fatty acids have been recognised, in particular their role as PG precursors. The majority of fatty acids consist of an aliphatic chain of C- atoms with a terminal carboxyl group. In some fatty acids not all free C-bonds are saturated with hydrogen (H) atoms, resulting in a double bond between two adjacent C-atoms (HC=CH); these are called unsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) contain two or more double bonds, normally in the cis configuration. Starting mainly from stearic acid (18:0) the body can produce a considerable number of long-chain PUFAs by the alternating action of a desaturase enzyme complex and an elongase enzyme system. These are liver microsomal enzyme complexes allowing the introduction of double bonds into fatty acid molecules. They require reduced pyridine nucleotide and molecular oxygen. They act only on activated fatty acids (acyl-CoA) according to the following reaction.

$$R_1 - CH_2 - CH_2 - R_2 - CoA + NAD(P)H + H^+ + O_2 \longrightarrow$$

$$R_1 - CH = CH - R_2 - CoA + NAD(P)^+ + 2H_2O$$

Endogenously formed PUFAs are members of the n-9 or n-7 families and since the introduction of double bonds and chain length addition occur at the carboxyl end of the fatty acid molecule there can be no interconversions

between the n-9 and n-7, and the n-3 and the n-6 families of fatty acids (although these interconversions occur readily in plants).

1.1.3 Essential fatty acid precursors

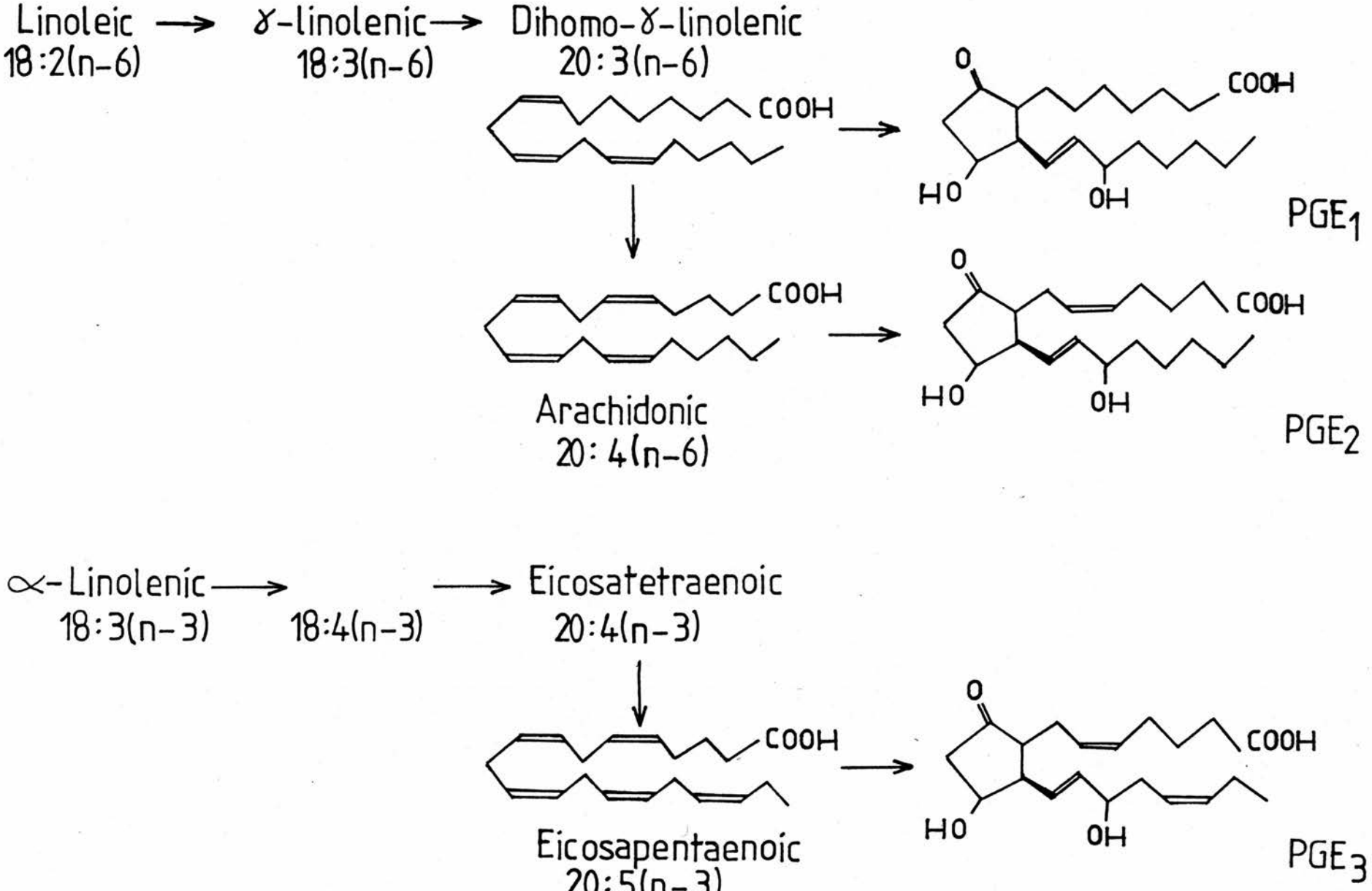
Saturated fatty acids and PUFAs of the n-9 and n-7 families are, on their own, insufficient to maintain normal life in mammals. In 1929, Burr and Burr observed that rats fed a rigidly fat-free diet, developed severe deficiency symptoms and ultimately died. This deficiency syndrome could be cured only by the administration of oils containing PUFAs of the n-3 and n-6 families. Similar results have been obtained in a variety of species including man. Mammals have an absolute requirement for n-3 and n-6 fatty acids because they lack $\Delta 12$ and $\Delta 15$ desaturases (present in plants) and therefore depend on dietary intake for these fatty acids, now termed essential fatty acids (EFAs).

In general, the EFAs are all cis C-20 unsaturated fatty acids with 3, 4 or 5 double bonds interrupted by methylene groups. Fatty acids are usually designated as a:b(n-c), where a is the total number of C-atoms, b is the number of double bonds and c is the position of the first double bond from the terminal methyl group. Three EFAs, dihomo-gamma-linolenic acid (DHLA) 20:3(n-6) ; arachidonic acid (AA), 20:4(n-6) and eicosapentaenoic acid (EPA), 20:5(n-3) are classical substrates for the biosynthesis of PGs of the 1, 2 and 3 series

respectively, as shown in Fig. 1.1. These EFAs are obtained from the meat and fish constituents of the diet. In animal tissues, these PG precursors can also be derived from linoleic acid (18:2(n-6)) and gamma-linolenic acid (18:3(n-6)) commonly found in the seeds and leaves of plants. First 18:2(n-6) is desaturated to 18:3(n-6) (also found in rare plant oils such as evening primrose oil), then 18:3(n-6) is rapidly chain elongated to 20:3(n-6) probably explaining why 18:3(n-6) is virtually undetectable in animal tissues. 20:3(n-6) is then avidly taken up into membrane and other phospholipid pools where it may then be liberated to serve as substrate for the synthesis of PGs. In most species including man, tissue phospholipid content of 20:3(n-6) is one third to one fiftieth that of arachidonic acid (20:4(n-6)) and since 20:4(n-6) and 20:3(n-6) are the precursors of the 2-series PGs and the 1-series PGs respectively, this probably explains why PGs of the 2- series predominate over PGs of the 1-series (Willis, 1981).

1.1.4 Structural lipids

Under normal circumstances, tissue free fatty acid levels are found at very small concentrations. Most fatty acids are present as triglycerides acting as a general reserve in adipose tissue or as membrane phospholipids performing a structural, or a metabolic function by providing PG precursors. Phospholipids have a glycerol backbone esterified in the 3 position to phosphoric acid. At the 1


Linoleic
18:2(n-6)
γ-linolenic
18:3(n-6)
Dihomo-γ-linolenic
20:3(n-6)
COOH
O
HO
OH
PGE1
Arachidonic
20:4(n-6)
PGE2
α-Linolenic
18:3(n-3)
18:4(n-3)
Eicosatetraenoic
20:4(n-3)
Eicosapentaenoic
20:5(n-3)
PGE3

and 2 positions are found hydrocarbon groups; at the 2 position largely a PUFA and at the 1 position generally a saturated fatty acid. The phosphoric acid groups form diesters with choline, ethanolamine, serine or inositol. For practical purposes sphyngomyelin is also considered as a phospholipid although it is in fact a sphyngosine derivative containing one amide-linked (mainly saturated or monounsaturated) long-chain fatty acid and a phosphorylcholine group attached to the terminal hydroxyl group of sphyngosine. Phospholipids can be hydrolysed by specific enzymes, phospholipase A_1, A_2, C or D, so-called depending on their site of action.

1.1.5 An historical coincidence

At about the same time as Burr and Burr discovered the essentiality of certain of the PUFAs, Kuzrok and Lieb (1930), who were studying artificial insemination, found that human semen produced both relaxation and potent contraction of the isolated human uterine strip. Later, Goldblatt (1933) and von Euler (1934) independently found that human seminal plasma and sheep seminal vesicle contain a potent lipid substance with smooth muscle-stimulating and vasodepressor activity, and which von Euler termed "prostaglandin" in the mistaken belief that it came from the prostate gland. Thereafter little definitive information on PGs was obtained until Bergstrom and associates (Bergstrom and Sjovall, 1957; Bergstrom, Eliasson, von Euler and Sjovall, 1959) isolated and synthesised pure PGs. As greater quantities

of pure PGs became available considerable progress was made towards elucidating the pharmacodynamic and biochemical action of each PG. Quite separately, information on the EFAs increased and it was only when the striking similarity in the molecular shapes of AA and PGE_2 was noted that it was discovered that PGs are synthesised from EFAs (Van Dorp, Beerthuis, Nugteren and Vonkeman, 1964; Bergstrom, Danielsson and Samuelsson, 1964).

1.2 PG synthesis

1.2.1 Source of precursor fatty acids.

PGs are not stored in cells but must be synthesised immediately prior to their release (Piper and Vane, 1971). The precursor fatty acids must be in a non-esterified form and are therefore not immediately available for PG synthesis but must be released from an intracellular lipid pool. The possible sources of precursor fatty acids are mono-, di- or tri-glycerides, cholesterol esters or phospholipids. Triglyceride fatty acids, particularly in adipose tissue, can be released by the action of a triglyceride lipase (Boyer, LePetit and Giudicelli, 1970) and in the ovary where there is a high concentration of cholesterol arachidonate, cholesterol esterase activity can be stimulated by luteinising hormone resulting in PG synthesis (Kuehl, 1974).

1.2.2 Phospholipases

The phospholipid fraction appears to be the major source of PG precursor fatty acids and most of the work on precursor release has concentrated on these lipids. The hydrolytic enzymes responsible for cleaving fatty acids are known as phospholipases. Phospholipases A_1 and A_2 (PLA_1 and PLA_2) release the fatty acids esterified in positions 1 and 2, respectively of the phospholipid molecule and phospholipase C hydrolyses the glyceryl-phosphate bond to liberate a phosphorylated base and a diglyceride. Phospholipase D is found mainly in plants and removes the base from the phospholipid to give phosphatidic acid (Fig. 1.2). As an unsaturated fatty acid is generally esterified in the 2 position of the phospholipid molecule, PLA_2 is an important enzyme in the regulation of PG synthesis. A phosphatidylinositol specific phospholipase C (in combination with a diglyceride lipase) may be one of the pathways responsible for AA release in response to platelet stimulation (Bell, Kennerly, Stanford and Majerus, 1979; Rittenhouse-Simmons, 1980).

1.2.3 Stimulus inhibition and turnover

A number of diverse stimuli, including mechanical stimulation, distension and tissue damage will initiate PG production via stimulation of phospholipases. Hormonal and other stimuli including antigen challenge, thrombin and collagen cause AA release from phospholipids by stimulating phospholipases. Thus phospholipases,

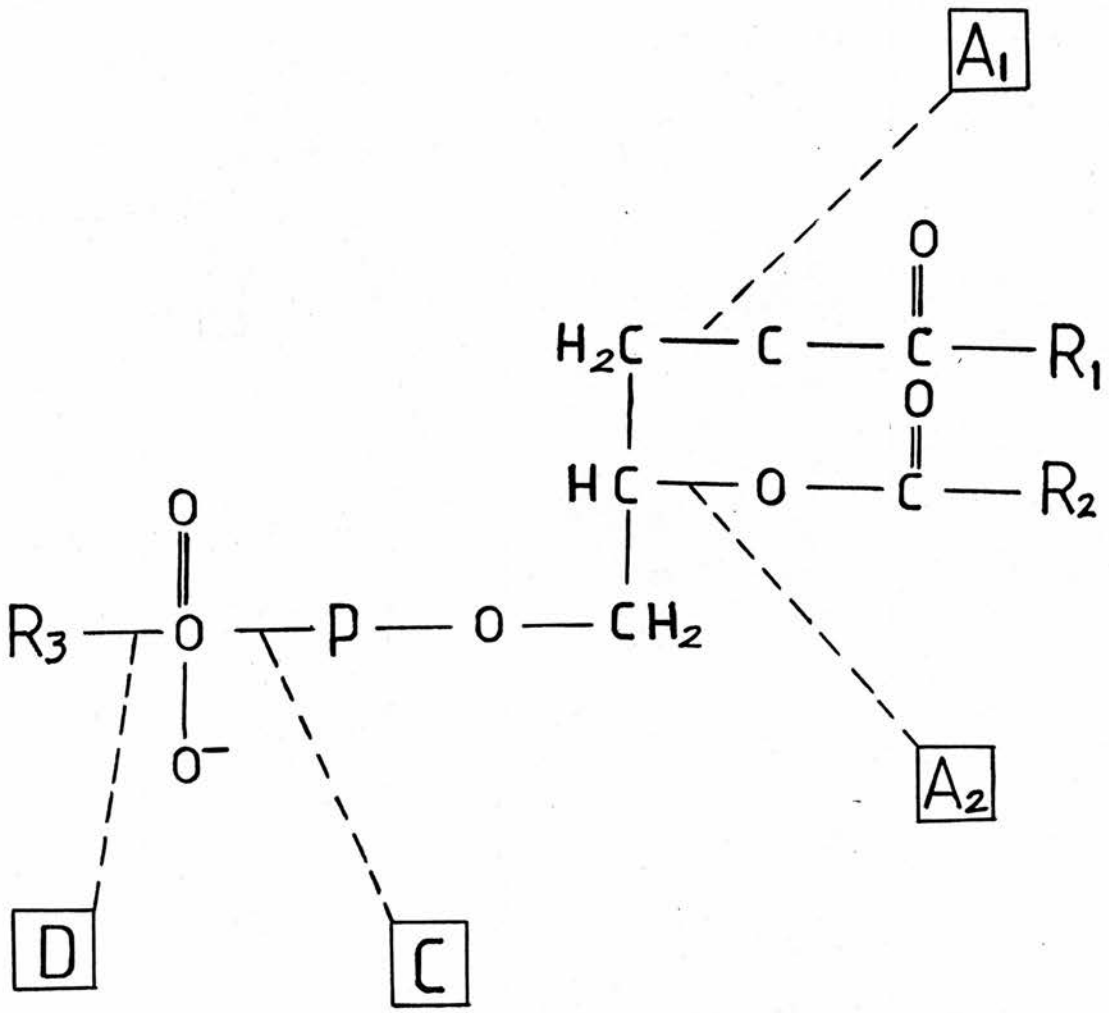


Fig. 1.2 Structure of phospholipid molecule. R_1 and R_2 fatty acid residues; R_3-alcohol group. A_1, A_2, C and D - site of action of respective phospholipases.

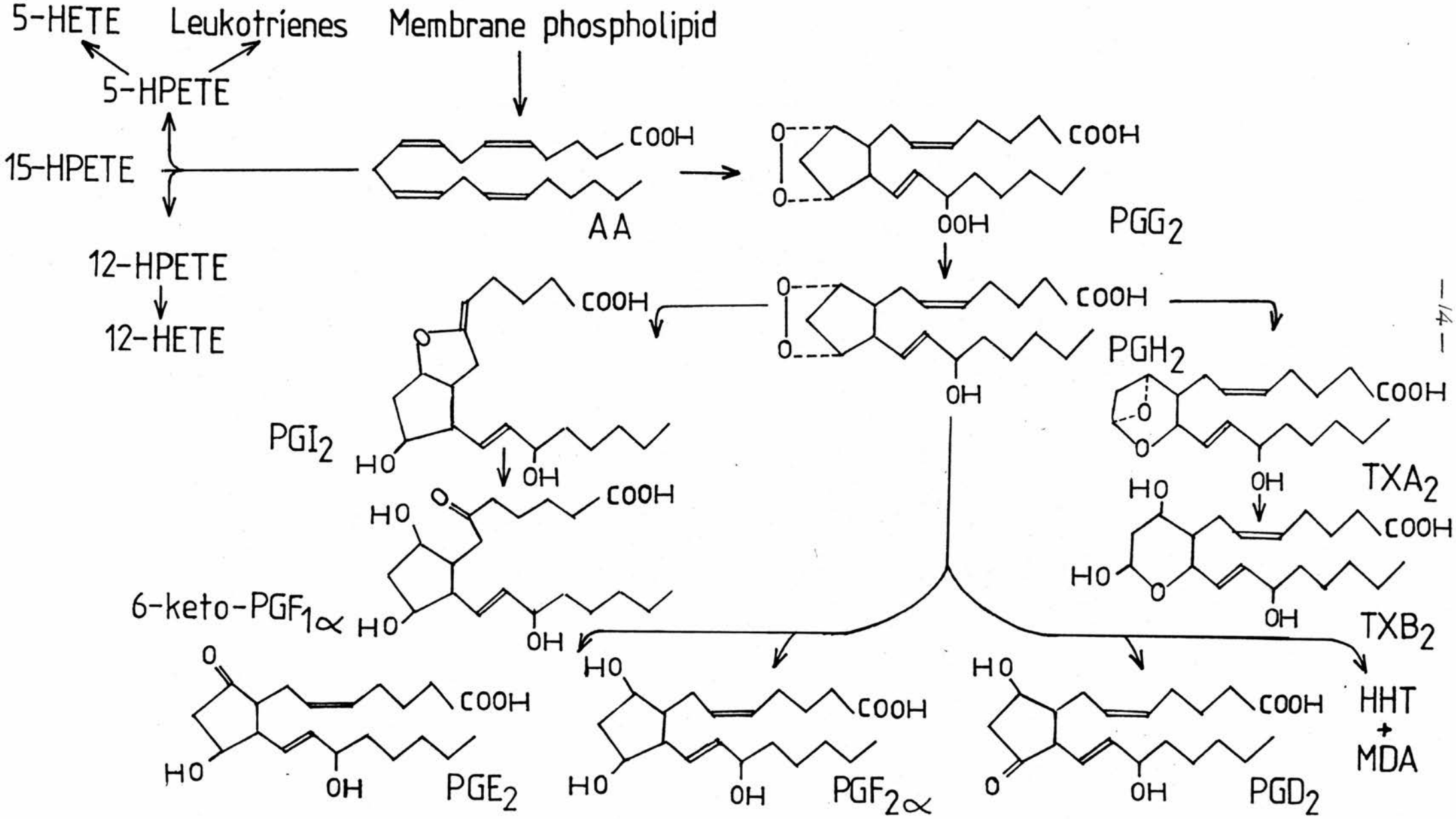
particularly PLA_2, provide the first possible target for controlling PG synthesis. Indeed, compounds such as mepacrine and chlorpromazine inhibit PLA_2 by forming complexes with phospholipids and preventing enzymic attack (Lullmann and Wehling, 1979). Polyamines such as putrescine and spermidine bind to the lipid surface of micelles and produce an identical effect to chlorpromazine. The activity of PLA_2 is dependent on Ca^{2+}, thus depriving the enzyme of Ca^{2+} is a very effective means of inhibition. It has been postulated that the calcium-binding protein calmodulin may be involved in phospholipase activation. Purified PLA_2 from snake venom, in the presence of Ca^{2+} was found to be stimulated by calmodulin (Moskowitz, Shapiro, Schook and Puszkin, 1983). In cultured porcine aortic endothelial cells, the stimulation of PGI_2 synthesis by bradykinin and the calcium ionophore A23187 was inhibited by TMB-8, an antagonist of intracellular calcium mobilisation, by the calcium channel blocker, methoxyverapamil, and by the calmodulin antagonists, W7 and trifluoperazine (Whorton, Willis, Kent and Young, 1984; Seid, MacNeil and Tomlinson, 1983). Calmodulin was found to stimulate the conversion of AA to TXB_2 in platelets and interestingly, was also found to inhibit the action of bovine lung 15-hydroxyprostaglandin dehydrogenase on ^3H-PGE_2 (Wong, 1981). Glucocorticoids have been known to interfere with PG generation for a number of years. These drugs have been found to act by inhibition of PLA_2 activity thus preventing the release of AA from membrane phospholipids (Gryglewski, Panczenko, Korbut, Grodzinska and

Ocetkiewicz, 1975; Hong and Levine, 1976; Blackwell, Flower, Nijkamp and Vane, 1978). Glucocorticoids have no direct effect on PLA_2 but appear to act by induction of proteins that possess anti-phospholipase properties (Danon and Assouline, 1978; Flower and Blackwell, 1979). A protein called macrocortin, with a molecular weight of 1500 daltons has been isolated from guinea-pig lungs and rat peritoneal macrophages and may be present in a pre-formed store in some cells. Rabbit neutrophils produce another protein, lipomodulin, (40000 daltons) which appears not to be stored and probably has to be synthesised *de novo* (Hirata et al, 1981; Blackwell, Carnuccio, Di Rosa, Flower, Langham, Parente, Persico, Russell-Smith and Stone, 1982). These inhibitory proteins are probably formed from the same precursor, and are now called lipocortins.

1.2.4 The cyclooxygenase pathway.

The next step in the synthesis of PGs is the insertion of molecular oxygen into AA. This reaction is catalysed by the enzyme cyclooxygenase (CO) and results in the formation of the PG endoperoxide, PGG_2 which is immediately converted by a peroxidase to PGH_2, with a sufficient amount of PGG_2 remaining at steady state to fully activate the CO under most conditions. The importance of hydroperoxides in PG biosynthesis was appreciated from early studies showing a decreased synthesis of PGs in the presence of glutathione and glutathione peroxidase (Lands, Lee and Smith, 1971).

Thus, in an autocatalytic reaction the CO activity forms the activating hydroperoxide, PGG_2. The work of Lands and colleagues (Lands, 1984) suggests that the self-limiting nature of the CO-catalysed reaction is due to enzyme self-inactivation rather than product inhibition. PGH_2 has a very short half-life because it is converted by various membrane bound enzymes into the different PGs and thromboxanes, a series of compounds characterised by an oxane instead of a cyclopentane ring. The activities of the different PG synthetases show quantitative differences in various tissues resulting in a different profile of products depending on which tissue is considered. The profile of possible AA products, usually known as the AA cascade is shown in Figure 1.3. A PGH_2-PGE_2 isomerase enzyme has been isolated (Ogino, Miyamoto, Yamamoto and Hayaishi, 1977) and very recently an enzyme catalysing the reduction of 9,11-endoperoxide PGH_2 to $PGF_{2\alpha}$ has been isolated from bovine lung (Watanabe, Yoshida, Shimizu and Hayaishi, 1985). The production of $PGF_{2\alpha}$ was previously believed to be largely a non-enzymic process. An enzyme, 9-keto-prostaglandin reductase, catalysing the reduction of PGE_2 to $PGF_{2\alpha}$ has been identified (Lands, 1979) and an enzyme, 9-hydroxyprostaglandin dehydrogenase, catalysing the reverse reaction has also been found (Pace-Asciak, 1975). The enzymes for the synthesis of TXA_2 and PGI_2, thromboxane synthetase and prostacyclin synthetase have been well characterised. The primary PGs, E and F are relatively stable and PGE_1 and $PGF_{1\alpha}$ were isolated in pure crystalline form comparatively early in the PG


5-HETE
Leukotrienes
Membrane phospholipid
5-HPETE
15-HPETE
12-HPETE
12-HETE
COOH
AA
OOH
PGG2
PGH2
OH
PGI2
HO
6-keto-PGF1∝
TXA2
TXB2
HHT
+
MDA
PGE2
PGF2∝
PGD2
O

field. However, the introduction of a modification of bioassay methods, the superfusion system, by Ferriera and Vane in 1967 was neccessary for the detection and subsequent characterisation of the labile and biologically active metabolites of arachidonic acid, such as TXA_2 and PGI_2. The PG endoperoxides, PGG_2 and PGH_2 were shown to induce platelet aggregation through the action of PGH_2 itself and also by its conversion to an unstable vasoconstrictor substance identified as TXA_2 (Hamberg and Samuelsson, 1974; Hamberg, Svensson and Samuelsson, 1975). However, TXA_2 was first described by Piper and Vane (1969) when they detected the release of an unstable substance, that contracted rabbit aorta, from sensitised guinea-pig lung challenged with antigen. This substance was called rabbit aorta contracting substance (RCS) and as its release was inhibited by indomethacin it was considered to be an arachidonic acid metabolite. It was later established that most of the activity of RCS could be ascribed to TXA_2. TXA_2 has a half-life at body pH and temperature of 30 seconds. The enzyme which synthesises TXA_2 from PG endoperoxides, thromboxane synthetase, was identified in the high-speed particulate fraction of human and horse platelets and has since been studied in some detail in human and bovine platelets (White and Glassman, 1976; Sun, 1977). TXA_2 is hydrolysed rapidly to TXB_2 which is considerably more stable than the parent compound and is the index of TXA_2 production most frequently measured. Prostacyclin, or PGI_2 was discovered in a similar manner to TXA_2 when the generation of an unstable substance with potent

anti-aggregatory properties was described in blood vessel microsomes stimulated with PGH_2 (Moncada, Gryglewski, Bunting and Vane, 1976). PGI_2 has a half-life of 2-3 minutes at body temperature and pH, becoming hydrolysed to 6-keto-$PGF_{1\alpha}$, a stable product.

1.2.5 The lipoxygenase pathway.

Another possible pathway from arachidonic acid is the lipoxygenase pathway producing hydroxy fatty acids from platelets, leukocytes and mast cells (Nugteren, 1975; Borgeat, Hamberg and Samuelsson, 1976) and leukotrienes, products with three conjugated double bonds and the major metabolites of AA in rabbit polymorphonuclear leukocytes (p.m.n.) (Borgeat and Samuelsson, 1979). In platelets, a hydroperoxy compound, 12 - hydroperoxy-5, 8, 10, 14-eicosatetraenoic acid (12-HPETE) is formed which is then converted to the hydroxy compound (12-HETE) Additional platelet lipoxygenase products are formed but their biological functions are not yet known.

Leukotrienes are formed from arachidonic acid by the 5-lipoxygenase pathway (Fig. 1.3). They have been found in lung tissue where they act as potent bronchoconstrictors and are thought to be involved in asthma and other hypersensitivity reactions (Walker, 1980; Hamberg, 1976). Leukotriene C has been identified as the smooth muscle contracting substance, first described by Feldberg and Kellaway (1938) and called

"slow reacting substance of anaphylaxis" (SRS-A), (Samuelsson, Borgeat, Hammarstrom and Murphy, 1980). Leukotriene B_4 is a potent chemotactic factor for human peripheral p.m.n. and rat peritoneal p.m.n. It is generated and released by various inflammatory cells after both physiological and pharmacological stimuli in vitro. LTB_4 is present in vivo, associated with acute, neutrophil-rich inflammatory lesions and it may be associated with certain pathological inflammatory states (for review see Bray, 1983).

1.2.6 PGs and cyclic nucleotides

Some effects of PGs are mediated through changes in the intracellular level of adenosine-3'-5'-monophosphate (cAMP), following binding of the PG to specific receptors at the surface of the target cells (Miller and Gorman, 1979). PGI_2 is a potent stimulator of platelet cAMP, considerably more potent than PGE_1 and also stimulates adenylate cyclase in isolated platelet membrane preparations (Gorman, Bunting and Miller, 1977). PGI_2 increases cAMP levels in cells other than platelets, including cultured human fibroblasts (Gorman, Hamilton and Hopkins, 1979) and guinea-pig lung homogenates (MacDermot, Barnes, 1980). PGI_2 and PGs of the E series have also been reported to increase intracellular cAMP levels in arterial smooth muscle in a dose-related manner by specific activation of adenylate cyclase (Beatty, Bocek and Young, 1973; Dembinska-Kiec, Rucker and Schonhofer, 1980). PGI_2 and PGs of the E series are

potent vasodilators in most vascular beds and relax the majority of vascular preparations in vitro (Vane, Bunting and Moncada, 1982). Increases in the intracellular levels of cyclic GMP (cGMP) or in the ratio of cGMP to cAMP have been associated with $PGF_{2\alpha}$-induced contraction of smooth muscle from dog lobar pulmonary and lateral saphenous veins (Kadowitz, Joiner, Hyman and George, 1975). Although the precise role for cGMP in smooth muscle contraction by PGs is not clear, there is evidence that unsaturated fatty acid peroxides contract smooth muscle concurrent with increased levels of cGMP (Gruetter and Ignarro, 1979; Asano and Hikada, 1979). Moreover, bradykinin-induced contractions of blood vessels were shown to be associated with increased levels of cGMP (Clyman, Sandler, Manganiello and Vaughan, 1975). Bradykinin may induce vasoconstriction in isolated bovine mesenteric veins by causing the accumulation of cGMP, which has been shown to increase the activity of 9-keto-prostaglandin-reductase, thus promoting the formation of $PGF_{2\alpha}$ from PGE_2 (Wong, McGiff and Terragno, 1977). From these findings it is possible to formulate the hypothesis that increased levels of cGMP are associated with constriction of blood vessels, and that the biological activities of the F series are related to the guanylate cyclase system. This is in contrast to the vasodilator actions of PGs of the E and I series which are related to the adenylate cyclase system.

1.2.7 Inhibition of cyclooxygenase

Vane (1971) and Smith and Willis (1971), independently and simultaneously reported that aspirin and indomethacin could inhibit PG synthesis in cell-free homogenates of lung tissue and human platelets, respectively. Vane (1971) showed that this effect was directly on the enzymatic conversion of AA to PGs. This observation led to an adequate explanation for the mode of action of such drugs in reducing inflammation, fever, hyperalgesia and platelet aggregation. This group of agents, the non-steroidal anti-inflammatory (NSAID) drugs provided a powerful research tool for the study of PGs. Several classes of inhibitor have now been identified (for review see Flower, 1974). Three types of inhibition are recognised; reversible competitive, reversible and irreversible non-competitive (Lands, 1981). Examples of reversible competitive inhibitors are fatty acids, closely related to the substrate with a comparable affinity, but not converted to oxygenated products. The precursor of the 3 series of PGs, EPA (20:5 (n-3)) is a poor substrate for CO and exerts an aspirin-like inhibitory effect on platelets (Needleman, Wyche, Le Duc, Sankarappe, Jakschik and Sprecher, 1981). The anti-inflammatory drug, ibuprofen, shows a similar binding for CO as AA (Rome and Lands, 1975). Aspirin covalently acetylates a lysine residue in the active site of the CO enzyme (Roth, Stanford and Majerus, 1975). Aspirin therefore inhibits the enzyme in platelets for the lifespan of these cells as platelets are unable to synthesise new protein (Steiner, 1970). Inhibition of vascular CO may persist for a much shorter period as new

enzyme may be generated. Also, the CO of human skin fibroblasts, arterial smooth muscle cells and human aorta appear to be less sensitive to inhibition by aspirin than human platelets (Baenzinger, Dillender and Majerus, 1977; Burch, Baenzinger, Stanford and Majerus, 1978).

1.3 Metabolism

1.3.1 General PG metabolism

There is no appreciable storage of PGs in tissues and the level of any PG released by a tissue will depend on a balance between synthesis and catabolism. Several enzyme systems which metabolise PGs have been isolated from different tissue preparations in vitro. The enzyme 15-prostaglandin hydroxydehydrogenase (15-PGHD) catalyses the oxidation of PGs at C-15 and is found in highest concentrations in lung, spleen and kidney (Larsson and Anggard, 1970) where it converts PGs to the 15-keto compound. Prostaglandin 13,14-reductase catalyses reduction of the 13 double bond of PGs and is found in highest concentrations in liver, spleen, small intestine and kidney although fairly high concentrations are found elsewhere in the body (Larsson and Anggard, 1970). PGs are also metabolised by β-oxidation of the carboxylic acid side chain and this has been demonstrated in rat liver mitochondria (Hamberg, 1968) and in homogenates of rat liver and kidney (Nakano and Morsy, 1971). β-oxidation results in the formation of dinor- and tetranor-derivatives of PGs. Enzymes catalysing ω-oxidation of the PG side chain are found in human

seminal plasma (Hamberg and Samuelsson, 1966) and guinea-pig and human liver microsomes (Israelsson, Hamberg and Samuelsson, 1969). Although thse early studies were carried out on the classical PGs more recent studies have shown that similar metabolic pathways exist for PGI_2. Namely that in rat, rabbit and monkey PGI_2 is metabolised via the 15-PGHD pathway to give the inactive or weakly active metabolites, 15-keto-PGI_2, 6,15-diketo-$PGF_{1\alpha}$ and 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$. PGI_2 is also metabolised by β-oxidation and 13,14-reductase pathways (Sun, Taylor, McGuire, Wong, Malik and McGiff, 1979). Similarly, in the cat an injection of PGI_2 resulted in a prominent peak of 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$ in plasma suggesting that metabolism is via the 15-PGHD, 13,14-reductase pathway (Machleidt, Foerstermann, Henning and Hertting, 1981).

1.3.2 Synthesis and metabolism of PGs by blood vessels

The enzyme 15-prostaglandin hydroxydehydrogenase (15-PGHD) is responsible for the catabolism of PGE_2 and $PGF_{2\alpha}$ and has been shown to be present in both arteries and veins (Wong and McGiff, 1977). PGI_2 can be metabolised by the 15-PGHD of bovine mesenteric arteries and veins, veins being more active than arteries, and is converted to 6,15 di-keto-$PGF_{1\alpha}$ (Wong, Sun and McGiff, 1978). In these tissues, 6,15 di-keto-$PGF_{1\alpha}$ was the only product recovered, probably reflecting the low levels of 13,14-reductase present.

1.3.3 Profile of PG metabolites in plasma and urine

In order to determine the possible physiological or pathological roles of PGs in humans and other species it is neccessary to have a reliable index of the production of each PG in either plasma or urine. For many years the 13,14, dihydro-15-keto PGs have been considered to be the optimal PG parameters in peripheral plasma. In the human, however, it was shown that after i.v. injection of [^{3}H] labelled $PGF_{2\alpha}$, the pattern of metabolites appearing with time in the circulation began to resemble the metabolic profile of products in the urine (Granstrom, Kindahl and Swahn, 1982). The major compound measured, the 11-ketotetranor metabolite, appeared to be present in the circulation for several hours, considerably longer than the 13,14, dihydro-15-keto metabolite. In a further study, Granstrom and Kindahl (1982) established that although the initial $PGF_{2\alpha}$ metabolite in cattle, rat and guinea-pig was 13,14, dihydro-15-keto $PGF_{2\alpha}$, the 11-ketotetranor product was the dominant plasma metabolite, whereas highly polar dicarboxylic acids dominated the metabolic profile in sheep and rabbit circulation. Five urinary metabolites of prostacyclin were identified by gas chromatography-mass spectrometry (GC-MS) in humans; the major compounds being the dinor metabolites of 6-keto-$PGF_{1\alpha}$ (Rosencranz, Fischer, Weimer and Frolich, 1980). A later study by Brash and co-workers showed that 82% of an administered dose of radio-labelled PGI_2 could be recovered in the urine and, in contrast to other species, only 4% in faeces. A number of metabolites

were identified by GC-MS, all retaining the 6-keto-$PGF_{1\alpha}$ structure and 6-keto-$PGF_{1\alpha}$ itself accounted for 6% of the administered dose (Brash, Jackson, Saggese, Lawson, Oates and Fitzgerald, 1983).

1.3.4 A metabolite of PGI_2 with biological activity, 6-keto-PGE_1.

In the last few years a considerable amount of interest has been shown in the synthesis and biological activity of 6-keto-PGE_1, a novel metabolite of PGI_2. 6-keto-PGE_1 was identified as a metabolite of PGI_2 in the Tyrode's perfused rabbit liver and its in vitro conversion from PGI_2 was demonstrated in cell-free homogenates of rabbit liver (Wong, Malik, Desiderio, McGiff and Sun, 1980; Wong, Lee, Quilley and McGiff, 1981). 6-keto-PGE_1 can be generated from PGI_2 or its hydrolysis product, 6-keto-$PGF_{1\alpha}$ by the action of 9-hydroxyprostaglandin dehydrogenase, an enzyme detected some years previously in rat kidney where it converts $PGF_{2\alpha}$ to PGE_2 (Pace-Asciak, 1975). 6-Keto-PGE_1 is produced by human platelets (Hoult, Lofts and Moore, 1981) and inhibits platelet aggregation in man and other species although its potency relative to PGI_2 varies from one study to the next. Originally reported as being equally as potent as PGI_2 (Wong, McGiff, Sun and Lee, 1979) other studies have shown it to be from 5 to 20 times less potent than the parent PG in inhibiting platelet aggregation (Lofts and Moore, 1982; Miller, Aiken, Shebuski and Gorman, 1980). Like PGI_2, 6-keto-PGE_1 appears to have identical

vasodepressor activity in rat or dog following either intravenous or intraarterial injection (Quilley, Wong and McGiff, 1979; Van Dam, Fitzpatrick, Friedman, Ramwell, Rose and Kot, 1981) suggesting that it also escapes pulmonary degradation in vivo. 6-keto-PGE_1 has potent effects on the cardiovascular system and is vasodepressor in all species studied, but again its potency relative to PGI_2 varies from one species to another and from one study to another. Its effect on blood pressure has been established in rat, rabbit, dog and cat and it is likely that this effect is on a number of vascular beds (Quilley et al., 1979; Van Dam et al., 1981; Hyman and Kadowitz, 1981). The discrepancies in potency of 6-keto-PGE_1 may reflect genuine species differences or perhaps a difference in the half life of 6-keto-PGE_1 in the circulation compared to PGI_2. The formation of 6-keto-PGE_1 from PGI_2 may be an important determinant of the actions of PGI_2 in vivo and may explain some of the discrepancies between the in vitro and in vivo actions of PGI_2; the apparently longer half-life of PGI_2 in plasma may be due to the greater stability of 6-keto-PGE_1 at physiological pH. However, the possibility of 6-keto-PGE_1 contributing to the actions of PGI_2 in man seems unlikely because studies of the metabolic disposition of PGI_2 have failed to detect metabolites with the 6-keto-PGE structure, and infusion of synthetic PGI_2 in volunteers does not result in accumulation of 6-keto-PGE_1 in plasma (Brash et al., 1983; Jackson, Goodman, Fitzgerald, Oates and Branch, 1982).

1.4 Platelet / vessel wall interaction

1.4.1 Vascular actions of the PGs

PGE_2 and $PGF_{2\alpha}$ have been shown to be produced by bovine aorta, mesenteric arteries and veins (Terragno, Crowshaw, Terragno and McGiff, 1975) and by bovine pulmonary artery and mesenteric artery of foetal and maternal origin (Terragno, McGiff, Smigel and Terragno, 1978). However, since the discovery of PGI_2 production by rabbit aorta microsomes (Moncada, Gryglewski, Bunting and Vane, 1976), PGI_2 has been presumed to be the major PG produced by blood vessels and the other PGs have been either ignored or assigned relatively minor roles in blood vessel function. There is evidence that in certain blood vessels, namely foetal ductus arteriosus, PGI_2 is not the principal product of AA metabolism (Terragno, Terragno, McGiff and Rodriguez, 1977) and in the microcirculation of the heart and brain, the major products of AA metabolism have been reported to be PGE_2 and PGD_2 respectively (Gerritzen and Printz, 1981). As mentioned above one of the first known actions of a PG was the potent vasodepressor effect of von Euler's lipid extract. Similar to vasoactive peptides and biogenic amines, PGs show considerable species and tissue differences in their pharmacological responses. It is well-established that PGs of the E-series are powerful vasodilators in many species of mammal, whereas $PGF_{2\alpha}$ is a moderately potent vasoconstrictor and/or pressor substance in dogs, rats and monkeys (Pike, Kupiecki and Weeks, 1967; Nakano and McCurdy, 1968; Du Charme, Weeks and Montgomery, 1968;

Weeks, Sekhar and Du Charme, 1969) but a vasodepressor in cats and rabbits (Anggard and Bergstrom, 1963; Horton and Main, 1963). PGI_2 is vasodepressor in dogs, rats and rabbits (Armstrong, Chapple, Dusting, Hughes, Moncada and Vane, 1977) and is considerably more potent than PGE_2. PGI_2 is a strong vasodilator in the mesenteric, hind-limb and pulmonary circulations of the dog (Dusting, Moncada and Vane, 1978; Kadowitz, Chapnick, Feigen, Hyman, Nelson and Spannhake, 1978).

1.4.2 PGs as circulating hormones.

These early studies on the systemic cardiovascular actions of PGs have provided the fundamental basis for the suggestion that these biologically active substances could be important in the regulation of blood pressure and blood flow. However, the investigations into the pulmonary metabolism of PGs by Vane and colleagues has neccessitated the modification of this hypothesis. Studies of the metabolism of PGs in the pulmonary circulation in vivo, showed that almost all of an infusion of PGE_1, PGE_2 or $PGF_{2\alpha}$ was inactivated in one passage through the lungs (Ferriera and Vane, 1967; Piper, Vane and Wyllie, 1970), but PGA_1 and PGA_2 survived passage through dog lung without being metabolised (McGiff, Terragno, Strand, Lee, Ng, 1969). The inactivation process for PGs was therefore very selective. Also, because the criterion for a circulating hormone required that a substance had to pass unchanged or even enhanced through the pulmonary circulation e.g.

angiotensin, (Ng and Vane, 1967) it was apparent that PGE_1, PGE_2 and $PGF_{2\alpha}$ could not be acting as circulating hormones. However, because PGA_2 was not destroyed by the lungs and a PGA compound was found to dilate blood vessels, lowering blood pressure and promoting salt and water excretion, it was suggested that PGA_2 could be the circulating renal anti-hypertensive substance counteracting those forces elevating blood pressure (Lee, McGiff, Kannegeisser, Aykent, Mudd, and Frawley, 1971). Using RIA, Zusman, Caldwell, Speroff and Behrman (1972) concluded that levels of PGA_2 in the plasma of normal man were greater than 1 ng/ml but other studies using mass-spectrometry, capable of detecting as little as 5 pg/ml of PGA_2, were unable to substantiate the presence of PGA_2 in plasma (Granstrom and Samuelsson, 1978). The material in plasma which cross-reacted with PGA-antibodies probably arose from non-enzymic degradation of PGE_2 during storage and sample preparation.

1.4.3 Is PGI_2 a circulating vasodepressor hormone?

Bioassay experiments suggested that PGI_2 produced by the lung acted as a circulating platelet inhibitory factor and a vasodepressor agent in rabbits and cats (Moncada, Korbut, Bunting and Vane, 1978; Gryglewski, Korbut, Ocetkiewcz, 1978). Using gas chromatography-mass spectrometry (GC-MS) measurements of plasma 6-keto-$PGF_{1\alpha}$ concentrations were made in patients undergoing cardiac catheterisation, the results suggesting that PGI_2 was

released from the lungs at a rate of 5ng/kg/min (Hensby, Barnes, Dollery and Dargie, 1979), strongly suggesting a role for PGI_2 as a circulating hormone in man. However, in another study by Fitzgerald, Brash, Falardeau and Oates (1981), the rate of PGI_2 secretion into the circulation was estimated by measurement (GC-MS) of 2 urinary metabolites of PGI_2 and was found to be approximately 0.1 ng/kg/min. Another study using GC-MS found that plasma levels of 6-keto-$PGF_{1\alpha}$ were less than 3 pg/ml (Blair, Barrow, Waddell, Lewis and Dollery, 1982). Similarly, neither human peripheral plasma, whole blood nor blood drawn directly from the heart contained any detectable PGI_2 (<20 pg/ml) as measured by gas chromatography with electron-capture detection of the pentafluorobenzyl derivative of 6-keto-$PGF_{1\alpha}$ (Christ-Hazelhof and Nugteren, 1981). Generally, RIA gives elevated levels of PGs compared with levels measured by GC-MS due to cross-reactions but two studies using RIA found systemic arterial and venous plasma levels of 6-keto-$PGF_{1\alpha}$ of < 50 pg/ml and approx. 7 pg/ml respectively (Edlund, Bonifin, Kaijser, Olin, Patrono, Pinca and Wennmalm, 1981; Forder and Carey, 1982) agreeing with the findings from GC-MS. Using a different approach, Steer, MacIntyre, Levine and Salzman (1980) established that addition of PGI_2 binding antibodies to platelet rich plasma (PRP) shortly after blood collection had no effect on platelet aggregation, and concluded that the circulating concentration of PGI_2 in humans was below that required for inhibition of aggregation. Pace-Asciak, Carrara and Levine (1981), using rats found that although

PGI_2 specific antibodies could sequester an administered dose of PGI_2, resting arterial blood pressure in normotensive and hypertensive rats was not significantly altered by anti-PGI_2 antibodies. Thus the evidence to date strongly suggests that PGI_2 is not acting as a circulating anti-platelet factor or as a circulating vasodepressor agent.

1.4.4 Platelet PGs and thromboxanes.

Kloeze (1967) showed that PGs had powerful effects on platelet aggregation in vitro; PGE_1 being strongly inhibitory whereas PGE_2 was slightly stimulatory. Later studies showed that PGD_2 and PGI_2 were potent antiaggregants. Smith and Willis (1970) demonstrated that stimulated platelets produce PGs themselves, in particular PGE_2. However, exogenous PGE_2 did not induce the platelet release reaction and aggregation, therefore a short-lived intermediate of AA conversion into PGE_2 was proposed. Indeed, two intermediates were found, the cyclic endoperoxides PGG_2 and PGH_2. Although the endoperoxides were able to aggregate platelets these studies suggested the presence of a highly unstable, potent endoperoxide derivative, which was confirmed in later studies and named thromboxane A_2 (Hamberg et al., 1975). The major effect of TXA_2 is the induction of platelet aggregation which can occur independently of the platelet release reaction (Kinlough-Rathbone, Reimers, Mustard and Packham, 1976; Meyers, Seachord, Holmsen, Smith and Prieur, 1979). TXA_2 is also a potent

vasoconstrictor and is probably involved in primary haemostasis (Mestel, Oetliker, Beck, Felix, Imback and Wagner, 1980).

1.4.5 Vascular PG synthesis

PGI_2 was first discovered in blood vessels (Moncada et al., 1976) and although it appears to be the major product of AA metabolism in vascular tissues, PGE_2 and $PGF_{2\alpha}$ are also produced by rabbit blood vessels and by porcine endothelial cells in culture (Foerstermann, Hertting and Neufang, 1984; Boeynams and Galand, 1983; Ody, Seillan, Russo-Marie and Duval, 1983). TXA_2 is produced in small amounts by human umbilical artery, by the pulmonary artery of the rabbit and by slices of piglet aorta and vena cava, although it is possible that TXA_2 is being produced by platelets contaminating these tissues. (Tuvemo,Strandberg, Hamberg and Samuelsson, 1976; Salzman, Salmon and Moncada, 1980; Siess, Dray, Seillan, Ody and Russo-Marie, 1981). However, TXA_2 has also been shown to be produced by vascular endothelial cells in culture (Ingerman, Aharony, Silver, Smith, Nissenbaum, Sedar and Macarak, 1980; Goldsmith and Needleman, 1982).

The actions of PGI_2 are largely the converse of TXA_2 in that it causes vasodilation, inhibits platelet aggregation and adhesion and stimulates platelet adenylate cyclase resulting in a marked increase in cAMP levels. The striking opposite effects of TXA_2 and PGI_2 on

platelet aggregation and blood vessel tone led to the hypothesis that a balance between the two compounds would regulate platelet aggregability and therefore determine arterial thrombosis tendency (Moncada and Vane, 1976). This hypothesis requires that vascular PGI_2 is able to reach the platelets and the initial studies on the possibility of PGI_2 as a circulating hormone seemed to validate this hypothesis.

1.4.6 Role of the endothelium in PGI_2 generation

The ability of the aorta to synthesise PGI_2 from exogenous PGH_2 is greatest at the endothelium and decreases progressively towards the adventitia (Moncada, Herman, Higgs and Vane, 1977). Studies using cultured vascular cells have also shown that endothelial cells are the most active producers of PGI_2 with smooth muscle cells in culture producing mainly PGE_2, although smooth muscle cells from rabbits and humans have been found to produce PGI_2 (Weksler, Marcus and Jaffe, 1977; MacIntyre, Pearson and Gordon, 1978; Ody and Duval, 1983; Siess et al., 1981; Larrue, Dorian, Demond-Henri and Bricaud, 1981; Baenzinger, Becherer and Majerus, 1979). Arteries appear to have a greater capacity for PG synthesis than veins as shown in rats and dogs (Skidgel and Printz, 1978; Eldor, Hoover, Pett, Gay, Alonso and Weksler, 1981). Cultured cells from human pulmonary arteries also produced greater amounts of PGI_2 than those from pulmonary veins, although this finding was not substantiated using fresh human arteries and veins

(Johnson, 1980; Moncada, Higgs and Vane, 1977). The possible importance of endothelial PGI_2 generation can be appreciated from studies where the endothelial cell layer has been removed. Following balloon de-endothelialisation of rabbit aorta the aortic surface was 87% covered by platelets. Infusion of PGI_2 completely inhibited platelet aggregation and reduced surface coverage by 84% (Adelman, Stemerman, Mennell and Handin, 1981). Tschopp and Baumgartner (1981) studied the effect of removing the aortic endothelium of different species and found that the degree of adhesion and aggregation of platelets to the sub-endothelium correlated negatively with the generation of PGI_2 in vitro by aortic segments of rat, rabbit and guinea-pig. PGI_2 synthetic capacity (from exogenous AA) was assayed at the surface of de-endothelialised rabbit aorta and was found to increase with time after injury, the increase in PGI_2 production correlating with the formation of a neointima (Eldor, Falcone, Hajjar, Minick and Weksler, 1981). De-endothelialisation of a small area of a branch of rat mesenteric artery, and subsequent superfusion with ADP, induced the formation of a thrombus which was markedly enhanced by the prostacyclin synthetase inhibitor, tranylcypromine (Bourgain, Andries, Biagi and Finne, 1981).

1.4.7 Endoperoxide steal hypothesis

Studies have shown that fresh vascular tissue can use both AA and PG endoperoxides for PGI_2 synthesis but that

the endoperoxides are the better substrate (Bunting, Gryglewski, Moncada and Vane, 1976). Also, vascular cells or endothelial cells treated with indomethacin incubated in platelet-rich plasma could generate a substance with PGI_2-like activity (Bunting et al., 1976; Gryglewski, Bunting, Moncada, Flower and Vane, 1976). From these studies it was concluded that the vessel wall is able to synthesise PGI_2 not only from endogenous precursors but also from PG endoperoxides released by platelets, implying that vessel wall PGI_2 synthesis may limit the extent of platelet reactions. Moncada and Vane (1978) proposed that this biochemical interaction between platelets and the vessel wall would continually prevent arterial thrombosis.

The hypothesis was challenged by several authors Needleman, Bronson, Wyche, Sivakoff and Nicolaou, (1978) showed that arachidonic acid was rapidly converted to PGI_2 by perfused rabbit heart and kidneys but that PGH_2 was not readily converted. Similarly, Hornstra, Haddeman and Don (1979) failed to find a significant difference in PGI_2 production of rat aorta incubated in platelet-rich (PRP) or platelet-poor plasma (PPP). Indomethacin-treated tissue did not produce any detectable amounts of PGI_2. However, it has been shown that when the platelet number is similar to normal blood levels, feeding of endoperoxides to endothelial cells suspended in platelet-rich plasma does occur (Marcus, Broekman, Weksler, Jaffe, Safier, Ullman and Tack-Goldman, 1981), although the relevance of such *in*

vitro experiments to *in vivo* events could be questioned. Of interest is the demonstration by Schafer, Crawford and Gimbrone (1984) that endoperoxides from uninhibited platelets were efficiently utilised by aspirin-pretreated bovine aortic endothelial cells for the formation of 6-keto-$PGF_{1\alpha}$, but that there was no evidence for the reverse transfer of endoperoxides from endothelial cells to platelets. More recent experiments have indicated an area in which "endoperoxide steal" could be of importance. Using selective thromboxane synthetase inhibitors several workers have shown a reduction in TXB_2 concentrations with concomitant increases in PGE_2 and $PGF_{2\alpha}$ (Smith and Gubiz, 1981), and PGI_2 concentrations (Randall, Parry, Hawkeswood, Cross, and Dickinson, 1981; Defryn, Deckmyn and Vermylen, 1982). More significantly, administration of thromboxane synthetase inhibitors to rats caused the redirection of PG production from TXB_2 to PGI_2 in an *in vivo* model when platelets were stimulated with collagen (Maguire and Wallis, 1984). Therefore the antithrombotic potential of thromboxane synthetase inhibitors is two-fold, reduction of TXB_2 production and the enhancement of vascular PGI_2 production.

As mentioned previously, it now seems unlikely that PGI_2 functions as a circulating hormone, and the phenomenon of agonist-specific desensitization of PGs on platelet adenylate cyclase would also preclude PGI_2 acting in the general circulation to prevent platelet aggregation (Miller and Gorman, 1979). However, it seems likely that

PGI_2 acts locally in the vasculature particularly where there is damage to the endothelium and therefore where there is platelet activation, thus limiting the extent of platelet aggregation. It is possible that in this situation platelet endoperoxides might provide an additional substrate for endothelial PGI_2 formation.

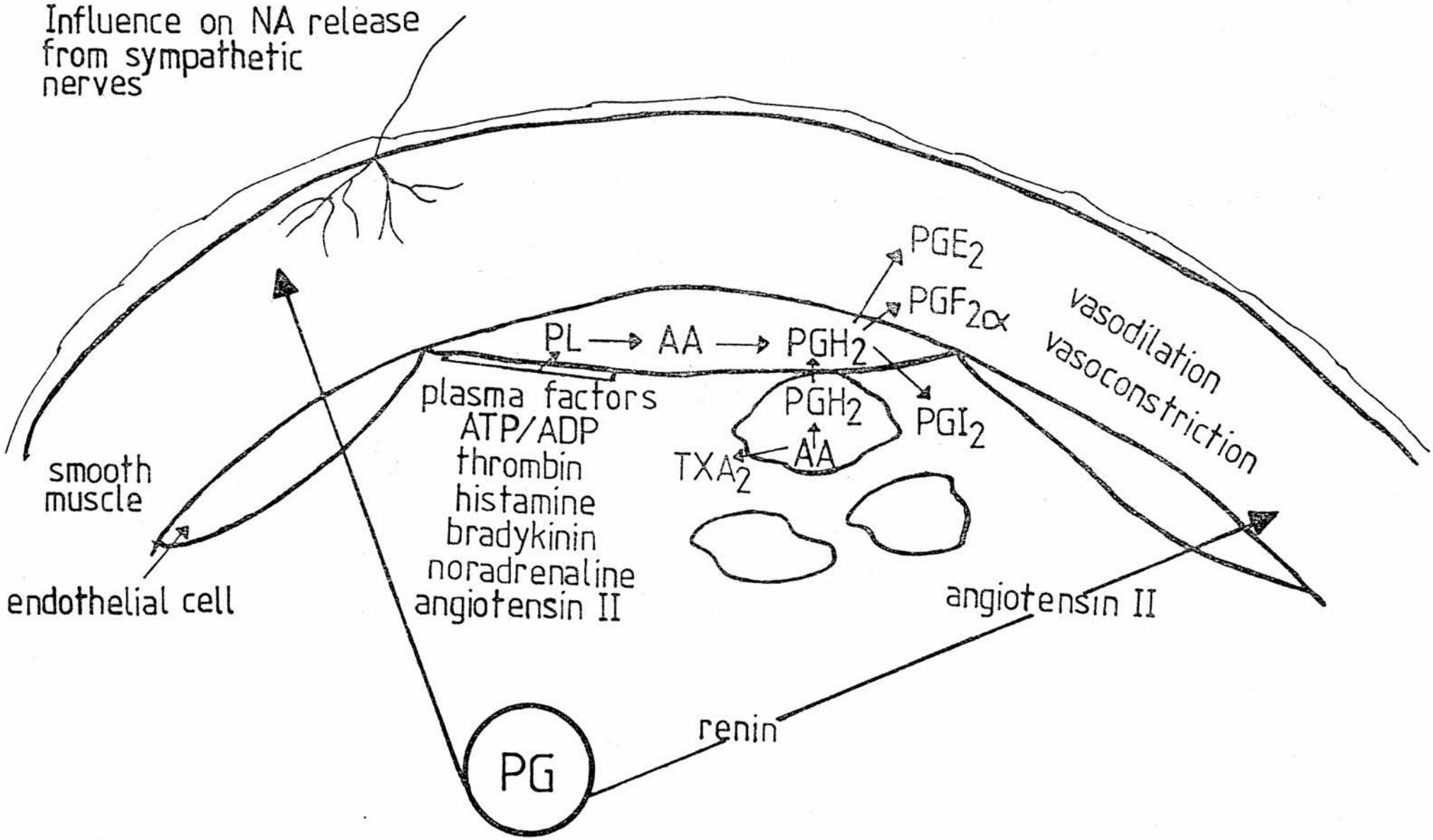
1.4.8 Aspirin as an antithrombotic agent

Early in vitro studies suggested that platelet CO was more sensitive to aspirin than that of vascular tissue and that, because of new enzyme synthesis by endothelium, vascular PGI_2 synthesis was restored more quickly than platelet TXA_2 synthesis (Burch et al., 1978; Jaffe and Weksler, 1979). Aspirin, therefore has the potential of being a valuable anti-thrombotic agent. However, segments of human vein incubated in vitro after patients had taken aspirin in vivo were inhibited from producing PGI_2 for at least 48 hr after doses of greater than 40 mg aspirin. TXA_2 production by platelets was inhibited 75% by a 40 mg dose of aspirin, additional doses producing a cumulative inhibitory effect on TXA_2 production (Weksler, Pett, Alonso, Richter, Stelzer, Subramanian, Tack-Goldman and Gay, 1983; Preston, Greaves and Stoddard, 1982).

Recently, it has been shown that repeated very low doses of aspirin (20 mg daily for 1 week) inhibit platelet TXA_2 formation by 93% and vascular PGI_2 production by 50%, indicating that there is also a cumulative effect of repeated small doses of aspirin (Weksler, Tack-Goldman, Karwande, Gay, 1983). These findings suggest that PG production by human vascular tissue is very sensitive to inhibition by conventional, fairly low doses of aspirin. Patrignani, Filabozzi and Patrono (1982) showed that 0.45 mg/kg per day of aspirin inhibited TXA_2 production by

platelets by >95% without any effect on urinary 6-keto-$PGF_{1\alpha}$ levels. Similarly, doses of aspirin of <160 mg/day were found to totally inhibit the urinary excretion of TXB_2 metabolites, while PGI_2 metabolites persisted at 25-40% of uninhibited values (Fitzgerald, Oates, Hawiger, Maas, Roberts, Lawson and Brash, 1983). These excretion studies are difficult to interpret as the precise origin of urinary PGs is not certain; an alternative interpretation of these findings could be that renal PG synthesis recovers more quickly from aspirin inhibition than vascular tissue from other sites. The clinical trials on the efficacy of aspirin in the prevention of myocardial infarctions and stroke have all been carried out using doses of 0.3 g/day or greater (1g/day as average). When the results of six randomised trials of aspirin in men who had survived a myocardial infarction were pooled, it appeared that aspirin was of modest benefit, reducing deaths by 16% and recurrent infarction by 21% (Prescott and Majerus, 1983). It now seems possible that similar results could be obtained using much lower doses of aspirin resulting in fewer toxic reactions.

Fig. 1.4 summarises some of the factors believed to be involved in the platelet / vessel wall interaction.


Influence on NA release from sympathetic nerves
PGE2
PGF2α
vasodilation
vasoconstriction
PL → AA → PGH2
plasma factors
ATP/ADP
thrombin
histamine
bradykinin
noradrenaline
angiotensin II
PGH2
PGI2
TXA2
AA
smooth muscle
endothelial cell
angiotensin II
renin
PG

1.5 Factors stimulating vascular PGI_2 production

1.5.1 Plasma factors

In 1978, MacIntyre, Pearson and Gordon observed that pig aortic endothelial cells were stimulated to produce PGI_2 by a factor present in cell free plasma. Similarly, a serum factor derived from human PRP or PPP stimulated 6-keto-$PGF_{1\alpha}$ release from rat aortic rings (Ritter, Orchard and Lewis, 1982). The stimulatory activity was heat-stable and was not inhibited by cycloheximide suggesting that its mechanism of action is not dependent on *de novo* protein synthesis. Seid, Jones and Russell (1983) established that both human plasma and serum could stimulate 6-keto-$PGF_{1\alpha}$ production by porcine aortic endothelial cells and that serum caused a significantly greater stimulation than plasma from the same individual. The supernatants from platelets stimulated with collagen also stimulated endothelial cell 6-keto-$PGF_{1\alpha}$ production. It is likely therefore that the plasma and serum stimulatory factor is derived from platelets.

1.5.2 ATP and ADP stimulation of PGI_2 production

Extracellular ATP is a potent vasoactive agent (Burnstock, 1981) requiring the presence of endothelial cells to exert its vasodilator effects (De Mey and Vanhoutte, 1981). Prostaglandins are released in biologically active concentrations from vascular beds perfused with ATP (Needleman, Minkes and Douglas, 1974) and it has since been shown that ATP and ADP dose

dependently stimulate the release of PGI_2 from cultured endothelial cell layers at concentrations (0.2-10.0 μm) that cause platelet aggregation, and which are reached in whole blood when platelets are activated (Pearson, Slakey and Gordon, 1983; Van Coevorden and Boeynaems, 1984; Ingerman-Wojenski, Smith and Silver, 1983). Extracellular ADP and ATP also stimulated the release of 6-keto-$PGF_{1\alpha}$ from rings of rat and rabbit aorta and rings of rabbit pulmonary artery (Boeynams and Galand, 1983).

1.5.3 Thrombin stimulation of PGI_2 production

Thrombin was found to stimulate the release of PGI_2 into the medium of human umbilical vein endothelial cells and bovine smooth muscle cells in culture (Weksler, Ley and Jaffe, 1978; Dejana, Balconi, Castellarnau, Barbieri, Vergara-Dauden and De Gaetano, 1983). However, although thrombin caused an endothelium-dependent relaxation of canine femoral artery rings, the relaxation was not affected by aspirin, indomethacin or the prostacyclin synthetase inhibitor, tranylcypromine (De Mey and Vanhoutte, 1981). Therefore the endothelium-dependent relaxation of canine femoral artery induced by thrombin is not mediated by PGI_2. In addition, the importance of the site of origin of endothelial cells used in these studies has been stressed. Goldsmith and Kisker (1982) demonstrated that in contrast to superfused umbilical veins where thrombin produced a 10-fold stimulation in the stimulation of 6-keto-$PGF_{1\alpha}$, sheep aortic vascular segments failed to release 6-keto-$PGF_{1\alpha}$ in response to

thrombin. Therefore, the demonstration that thrombin induces the release of PGI_2 from umbilical vein endothelial cells may not be representative of the responses of other parts of the vasculature. The production of thrombin at a site of vascular injury could, by stimulating PGI_2 synthesis by endothelial cells adjacent to the injured area limit the extent of the platelet response and help to localise thrombus formation.

1.5.4 Angiotensin stimulation of PGI_2 production

Several authors have described a stimulatory effect of the potent vasoactive agent, angiotensin II (AII) on the release of PG-like material either *in vivo* or in perfused organs (McGiff, Crowshaw, Terragno and Lonigro, 1970; Dusting, Mullins and Nolan, 1981). Grodzinska and Gryglewski (1980) showed that AII perfused through rabbit mesenteric artery and guinea-pig lungs induced the release of prostacyclin and that this effect was mediated through AII receptors. Nolan, Dusting and Martin (1981), found that the release of PGI_2 in response to AII in the isolated rat mesenteric bed was reduced by dexamethasone and mepacrine, compounds both reported to inhibit phospholipase A_2. Stimulation of PGE-like material by AII has also been reported for human umbilical vein endothelial cells in culture (Gimbrone and Alexander, 1975), although a later study was unable to demonstrate any effect of AII on PG formation in human umbilical artery or vein endothelial cells (Alhenc-Gelas, Tsai, Callahan, Campbell and Johnson, 1982).

1.5.5 Bradykinin stimulation of PGI_2 production

Bradykinin is a potent vasodilator and hypotensive agent although it has opposing effects on arteries and veins, dilating arteries and constricting veins (Bobbin and Guth, 1968). Its activity may be mediated, at least in part, by PGs. Early studies demonstrated that bradykinin could induce the release of PGs (specifically PGE_2 and PGI_2) from isolated, perfused rabbit kidneys, hearts and mesenteric blood vessels, and *in vivo*, from the rabbit ear artery (Isakson, Raz, Denny, Wyche and Needleman, 1977; Blumberg, Denny, Marshall and Needleman, 1977; Simmet and Herting, 1980; Sametz and Juan, 1982). In the anaesthetised dog, PGI_2 release induced by bradykinin was mainly of renal origin. The sustained hypotensive effect of bradykinin was reduced after inhibition of PG synthesis with indomethacin, suggesting that PGI_2 released in response to bradykinin was involved in the blood pressure response (Mullane and Moncada, 1980). However, the venoconstrictor action of bradykinin may depend on the capacity of the vein to increase its production of $PGF_{2\alpha}$, as shown by abolition of the bradykinin-induced contraction of bovine mesenteric vein after indomethacin treatment (Wong, Terragno, Terragno and McGiff, 1977). Later studies have established that bradykinin can stimulate the release of [^{3}H] AA from labelled porcine aortic endothelial cells in culture (Whorton, Young, Data, Barchowsky and Kent, 1982), and can stimulate the release of PGI_2 from endogenous AA in these cells (Pearson, Carleton and Hutchings, 1983). Bradykinin selectively stimulated the release of

6-keto-$PGF_{1\alpha}$ from human umbilical vein endothelial cells in culture without significantly affecting the release of PGE_2, $PGF_{2\alpha}$ or TXB_2; but bradykinin stimulated the release of both 6-keto-$PGF_{1\alpha}$ and PGE_2 from umbilical artery endothelial cells (Alhenc-Gelas et al., 1982) In addition, bradykinin enhanced the release of PGE_2 (as well as PGI_2) in pulmonary artery. The release of PGI_2 from bovine pulmonary artery endothelial cells stimulated by bradykinin was abolished by the Ca^{2+} chelator, EGTA, by the slow calcium channel antagonists, verapamil and nifedipine and by the calmodulin inhibitor, trifluoperazine, suggesting that bradykinin mediates its effects on PGI_2 formation by activation of a phospholipase via a Ca^{2+}-calmodulin dependent pathway (Crutchley, Ryan, Ryan and Fisher, 1983).

1.5.6 Histamine stimulation of PGI_2 production

An injection of histamine into the isolated perfused rabbit ear dose-dependently stimulated the release of PGE_2 and PGI_2. In arteries pre-labelled with [^{14}C] AA histamine stimulated the release of radioactive AA and increased the release of PGI_2, PGE_2, $PGF_{2\alpha}$ and PGD_2. The H_1 receptor antagonist mepyramine abolished the histamine-stimulated release of PGs (Juan and Sametz, 1980). Histamine also stimulated PGI_2 formation in cultured human umbilical vein endothelial cells (Baenzinger, Force and Becherer, 1980) but did not have a significant effect on venous and arterial smooth muscle cells or skin fibroblasts (Baenzinger, Fogerty, Mertz and Chernuta, 1981). Another study by Alhenc-Gelas, Tsai,

Callaghan, Campbell and Johnson (1982) has shown that human arterial and venous umbilical endothelial cells in culture synthesise $PGF_{2\alpha}$ and PGE_2 as well as PGI_2 and that histamine stimulates not only PGI_2 release but also $PGF_{2\alpha}$ and PGE_2 release from venous cells. The release of PGs induced by histamine was inhibited by the H_1 receptor antagonist, pyrilamine but not by the H_2 receptor antagonist, cimetidine.

1.6 Other factors influencing vascular PG production

1.6.1 Nutritional factors

If a deficiency of vasodilatory PGs initiates or aggravates hypertension, then inhibition of PG production by dietary deprivation of precursor fatty acids should result in elevated blood pressure. The importance of the availability of PG precursors such as linoleic acid can be appreciated from several studies. Dyerberg and co-workers (Dyerberg, Bang, Stofferson, Moncada and Vane, 1978) were the first to suggest that the low incidence of coronary heart disease among Eskimos may be due, at least in part, to their high intake of EPA leading to an anti-thrombotic state in which an active PGI_3 and an inactive TXA_3 are formed. Thus EPA could act in two ways to reduce platelet aggregability; it competes with AA for platelet cyclooxygenase but is itself a poor substrate for the enzyme, reducing TXA_2 formation; TXA_3 formed is not aggregatory (Fischer and Weber, 1983). PGI_3 ($\Delta 17$-PGI_2), has been synthesised from PGH_3 by aortic microsomes and has been shown to have properties very

similar to PGI_2, although it has not been shown that vessel wall can convert EPA to PGI_3 (Needleman, Raz, Minkes, Ferrendelli and Sprecher, 1979). In 1974, Rosenthal, Simone and Silbergleit first described an increase in systolic blood pressure in the rat during dietary deprivation of PG precursors and although this finding was later confirmed by other workers (Triebe, Block and Forster, 1976), the mechanism of the increase in blood pressure was not clear. These studies did show however, that a linoleic acid-deficient diet augments the development of hypertension in rats with a high salt intake while a linoleic acid-enriched diet attenuates the hypertension. Further studies established that the linoleic acid content of the diet had little effect on the development of salt-induced hypertension in the rat if the salt load was excessively high, but if the salt-load was more moderate then a low linoleic acid content exaggerated the hypertension (Box and Mogenson, 1980; MacDonald, Kline and Mogenson, 1981). In contrast, in the spontaneously hypertensive rat (SHR) a high level of dietary linoleic acid was associated with higher blood pressures than a low level of linoleic acid in the diet (Box and Mogenson, 1982). A more detailed study established that blood pressure increased significantly in rats fed a linoleic acid-deficient diet and that generation of PGI_2-like activity from segments of aorta in vitro was suppressed compared to rats fed a 5 or 9 en% linoleic acid diet (Dusing, Scherhag, Glanzer, Budde and Kramer, 1983). In comparison with diets rich in linoleic acid which might be expected to enhance PG synthesis, the

feeding of diets rich in n-3 fatty acids such as linolenic acid and EPA decreases PG production (Ten Hoor, deDeckere, Haddeman, Hornstra and Quadt, 1980) and is associated with an increase in blood pressure of normal rats (Scherhag, Kramer and Dusing, 1982).

1.6.2 Gender differences in PG production

The mortality and morbidity from cardiovascular diseases is much greater in males than in pre-menopausal females (Kuller, 1976). Male and female differences in the production of PGs have been reported, with male rats having been found to release more 6-keto-$PGF_{1\alpha}$ from aortic rings than female rats (Pomerantz, Maddox, Maggi, Ramey, Ramwell, 1980). Furthermore, male lungs converted AA into 6-keto-$PGF_{1\alpha}$ more readily than female lungs (Maggi, Tyrell, Maddox, Watkins and Ramey, 1980) and microsomes of kidney medulla from male rats produced greater amounts of PGD_2, PGE_2 and $PGF_{2\alpha}$ than those from female rats (Gesce, Ottlecz, Schaffer, Bujdosc and Telegdy, 1979). The sex steroids have been implicated in these differences in AA metabolism in blood vessels. Experimentally-induced arterial thrombosis is markedly enhanced by testosterone treatment in male and female rats, whereas oestradiol significantly increased the obstruction time and decreased the thrombus weight in male, but not female rats (Uzonova, Ramey, Ramwell,1976). If vascular PGI_2 were involved in these effects on thrombosis via its anti-platelet actions, as suggested by the work of Ohtsu and colleagues (Ohtsu, Saitoh, Okada,

Chang and Murota, 1983) where oestrogen treatment was shown to inhibit artificially induced thrombus formation with the concomitant stimulation of PGI_2 synthesis in the aorta, then females might be expected to show a greater production of PGI_2 than males. However, this is not the case as evidenced from the findings of Maggi et al. (1980) and Pomerantz et al. (1980). *In vitro*, oestrogen has been shown to stimulate PGI_2 production in cultured rat aortic smooth muscle cells (Chang, Nakao, Orimo, Murota, 1980) and cultured porcine endothelial cells (Seillan, Ody, Russo-Marie and Duval, 1983). A combined oestrogen/progestagen treatment stimulated PGI_2 release from aortic rings and from segments of aorta, vena cava, heart and lung (Karpati, Chow, Woollard, Hutton and Dandona, 1980; Roncaglioni, de Minno, Rayers, de Gaetano and Donati, 1979). In contrast, testosterone treatment of male rats significantly inhibited the production of PGI_2 by rings of aorta (Chang, Nakao, Tai and Murota, 1982) and either inhibited (Nakao, Chang, Murota and Orimo, 1981) or had no effect on PGI_2 production by rat or pig aortic smooth muscle cells in culture (Pomerantz et al., 1980; Seillan et al., 1983). There was no effect of testosterone treatment on the release of 6-keto-$PGF_{1\alpha}$ from aortic rings of adult rats castrated at birth (Wey, Skjaerlund and Subbiah, 1983).

1.6.3 Effects of age on PG production

A complex interrelationship exists between blood pressure and arterial disease, hypertension increasing the

susceptibility of blood vessels to arteriosclerosis. It is generally accepted that in humans, blood pressure increases with age as does the incidence of coronary heart disease and thrombosis. Studies to date do not show unequivocally that these increases in blood pressure and thrombotic tendency with increasing age are related to PG mediated effects. It has been shown that 15-hydroperoxyarachidonic acid (15-HPAA), a lipid peroxide, is a potent and selective inhibitor of PGI_2 generation by porcine vessel wall microsomes or by fresh vascular tissue (Moncada et al., 1976). Other fatty acid peroxides and their methyl esters show similar effects (Salmon et al., 1978). Lipid peroxidation induced by free radical formation is known to occur in the ageing process (Slater, 1972) and it is reasonable to suggest that PGI_2 generation could be reduced in aged blood vessels because of the effects of greater concentrations of lipid peroxides.

Pace-Asciak and Carrara (1979) and Panganamala and co-workers (Panganamala, Hanumaiah and Merola, 1981) have shown that PGI_2 formation by aortic homogenates or intact aorta increases with age in the rat. However, these authors were studying a very limited age range using rats of up to 20 weeks of age and were not looking at the situation in the old animal. Aortic smooth muscle cells from old rats produced less PGI_2 in culture than those obtained from young rats and this was due to a specific decrease in PGI_2 synthetase activity. These cells from old rats were found to produce more PGE_2 than PGI_2 (Chang

et al., 1980). Using bovine smooth muscle and endothelial cells in vitro (Ager et al., 1982) it was observed that during sub-culture the ability to generate PGI_2 decreased while PGE_2 formation increased. It is not known if these changes are due to specific effects of lipid peroxides on PGI_2 synthetase.

1.6.4 PG production in disease states

A number of diseases have now been related to an imbalance in the PGI_2/TXA_2 system. Platelets from patients with arterial thrombosis or deep vein thrombosis produce greater amounts of PG endoperoxides and TXA_2 than healthy controls and have a shortened survival time (Lagarde and Dechavanne, 1977). Platelets from rabbits made atherosclerotic by feeding a high cholesterol diet and from patients who have survived myocardial infarction are abnormally sensitive to aggregating agents and produce more TXA_2 than controls (Shimamoto, Kobayashi, Takahashi, Takashima, Sakomoto and Morooka, 1978; Szczeklik, Gryglewski, Musial, Grodzinska, Serwonska and Marcinkiewicz, 1978). Diabetic patients are highly susceptible to microvascular complications, atherosclerosis and thrombosis and it has been shown that platelets from rats made diabetic by streptozotocin released more TXA_2 than controls, whereas the aorta from these rats showed a reduced release of PGI_2 (Harrison, Reece and Johnson, 1978). Similarly, vascular PGI_2 production in diabetic patients was depressed and circulating levels of 6-keto-$PGF_{1\alpha}$ were reduced in

diabetic patients with proliferative retinopathy, although there did not appear to be an association between reduced PGI_2 production and diabetic retinopathy (Johnson, Harrison, Raftery andd Elder, 1979; Dollery, Friedman, Hensby, Kohner, Lewis, Porta and Webster, 1979; Davis, Brown, Finch, Mitchell and Turner, 1981).

PGI_2 generation is reduced in arteries from rabbits with experimental atherosclerosis and in human atherosclerotic plaques (Dembinska-Kiec, Gryglewska, Zmuda and Gryglewski, 1977; D'Angelo, Villa, Mysliwiec, Donati and de Gaetano, 1978). In early experimental atherosclerosis in rabbits it has been found that platelets have an increased sensitivity to the antiaggregatory effect of PGI_2, an increased sensitivity to ADP and increased TXA_2 formation (Gryglewski, Dembinska-Kiec, Zmuda and Gryglewska, 1978; Dembinska-Kiec, Rucker and Schonhofer, 1979). This probably reflects a decreased in vivo PGI_2 concentration resulting in an increased sensitivity of the platelets to PGI_2. Cultured smooth muscle cells from atherosclerotic rabbit aorta also produce decreased amounts of PGI_2 (Larrue, Rigaud, Daret, Demond, Durand and Bricaud, 1980).

1.7 Hypertension

An exhaustive review of the possible causes of hypertension and its attendant pathologies is beyond the scope of this discussion, but the basic findings will be outlined and in particular the possible involvement of

PGs in the aetiology of the disease. In both clinical and experimental hypertension the final common pathway leading to an elevated arterial pressure is an increased total peripheral resistance. Structural abnormalities in the vasculature that characterise the hypertensive process are (1) changes in the vascular media, (2) rarefication of the resistance vessels, (3)lesions of the intimal surface. These structural changes result in an altered physical state on which hormones, such as noradrenaline and angiotensin II, can act. Hypertension is a multifactorial disorder in which a number of physiological mechanisms participate to elevate arterial pressure; these include intravascular volume changes involving the kidney, steroid hormones, sodium and water balance and other pressor mechanisms such as the renin-angiotensin-aldosterone system; altered catecholamine metabolism and neural function; other hormonal influences and possibly the two depressor mechanisms, the kallikrein-kinin and prostaglandin systems. A variety of clinical and experimental forms of hypertension have been described and intensively studied involving renal pathology, excessive production of hormones or altered catecholamine metabolism. However, probably over 95% of patients with hypertension have essential hypertension, i.e. hypertension of undetermined cause which seems to have a genetic predisposition and is slowly progressive with regard to the vascular and organ involvement. Therefore, an experimental model mimicking this condition must also be naturally occuring, predisposed genetically and slowly progressive. There are

now available several experimental models of essential hypertension and these will be discussed in greater detail.

1.7.1 Animal models of hypertension - the genetically hypertensive rat

Hypertension can be induced in animals by several methods and there are now available a number of models of hypertension, using the rat. Experimentally, hypertension can be induced by clipping the renal artery to produce two forms of renovascular hypertension (Goldblatt hypertension); one form, consisting of one clipped kidney with the opposite kidney intact, is associated with high renin levels and relatively reduced intravascular volumes whereas the second form, consisting of one clipped kidney with the opposite kidney removed is associated with higher volumes and consequently relatively lower renin levels. Hypertension can also be induced by salt-loading animals treated with the mineralocorticoid, deoxycorticosterone acetate. However, for an animal model to represent the most common form of human hypertension, essential hypertension, it is required to be polygenic in origin and influenced by environmental factors. Two strains of rat fulfilling these criteria have been bred through brother to sister mating, the spontaneously hypertensive rat (SHR) of Okamoto (Okamoto and Aoki, 1963) and the New Zealand genetically hypertensive (GH) strain of Smirk (Smirk and Hall, 1958).

(For comprehensive reviews on the validity of using genetically hypertensive rats in the study of hypertension see McGiff and Quilley, (1981) and Trippodo and Frolich, (1981)).

Other hypertensive strains are now available but appear to involve primarily alterations in renal, sodium and water metabolism (Milan strain), high sensitivity to sodium intake (Dahl strain) and the Lyon strain (Vincent, Bornet, Berthezene, Dupont and Sassard, 1978). The New Zealand GH strain appears to be most similar to the Japanese SHR but has not been studied in as great detail. The relevance of using a genetic model of hypertension in the rat will be discussed largely with reference to the SHR as the majority of studies have been conducted using this animal. It is probable that many of these findings also apply to the GH rat.

Of course there are several important differences between the SHR and essential hypertension in humans; the SHR (and GH rat) usually weigh less than their normotensive control whereas the clinical hypertensive population seem to be heavier than controls; thyroid function may be altered in SHR but not all hypertensive patients; SHR show inconsistent responses to commonly used anti-hypertensive agents and in general the rat is relatively resistant to atherosclerosis. This is in marked contrast to the facilitative effect of an elevated arterial pressure on the development of atherosclerosis in hypertensive man (Hollander, 1976). Another criticism

of the SHR is that its control strain, the Wistar Kyoto (WKY) was not developed simultaneously. This also applies to the GH rat and its Wistar control. The use of the rat as an experimental animal when studying the role of PGs in hypertension was challenged when it was discovered that PGE_2, a major renal PG and potent vasodilator, constricted the vasculature of the isolated rat kidney, an effect not observed in any other species examined (Malik and McGiff, 1975). The major conclusion of this study was that PGs of the E series could contribute to the elevation of blood pressure in the rat i.e. PGE_2 was pro-hypertensive in the rat but anti-hypertensive in the rabbit, and man and other species resembled the rabbit in this respect. However, because of the importance of renal mechanisms in the regulation of blood pressure, the anomalous effects of PGE_2 in the rat probably need only occur in the kidney to cause elevation of blood pressure. Indeed, there is evidence that PGE_2 dilates other vascular beds of the rat (Gerber and Nies, 1979). The constrictor response of the renal vasculature of the rat to PGE_2 was found to be exaggerated in the GH strain and PGs released by the action of noradrenaline (NA) were found to augment the renal vasoconstrictor response to NA. An important finding in relation to the use of the GH strain in studies of hypertension was the deficiency of the major catabolising enzyme 15-PGHD, a defect leading to higher levels of PGE_2 intrarenally (Armstrong, Blackwell, Flower, McGiff, Mullane and Vane, 1976). Thus, the importance of increased renal PGE_2 levels in the GH rat can be appreciated from a consideration of

PG-adrenergic interactions, whereby increased levels of a modulator such as PGE_2 will, in the rat potentiate the effects of the sympathetic nervous system. With these important caveats in mind the genetically hypertensive rat has many characteristics in common with the human form of essential hypertension, and to date is the best model available for this form of hypertension.

1.8 PGs in hypertension

1.8.1 Vascular actions of PGs

A large number of studies have investigated the possible involvement of PGs in the aetiology of hypertension in man and animal models. There is evidence that PGs are involved in (i) the mechanisms regulating vascular resistance; (ii) the pressor and depressor responses to vasoactive hormones; (iii) renin secretion and renal handling of sodium and water. A defect in the production of certain PGs could result in a reduction in vasodilator capacity with the consequent increase of vascular resistance, associated with a reduction of renin secretion (Case, Casarella, Laragh, Fowler and Canon, 1978), and increased responsiveness to noradrenaline (NA) and angiotensin II (AII) at normal endogenous levels of these pressor hormones (Philipp, Distler and Cordes, 1978; Weidmann, 1980). The other aspect of a defect in PG production in hypertension is seldom considered, namely the possibility of an increased production of the vasoconstrictor AA metabolites, $PGF_{2\alpha}$ and TXA_2.

There have been many attempts to determine the involvement of PGs in the control of blood pressure by the administration of cyclooxygenase inhibitors. In the anaesthetised dog, the immediate haemodynamic effects of indomethacin resembled those of uncomplicated essential hypertension: elevated blood pressure, unchanged cardiac output, a marked increase in renal vascular resistance and a smaller increase in that of the limbs (Lonigro, Itskovitz, Crowshaw and McGiff, 1973). Daily administration of indomethacin was reported to elevate mean arterial pressure in the rabbit without altering plasma volume or increasing sodium retention (Colina-Chourio, McGiff and Nasjletti, 1974). In addition, in normal and in sodium-depleted subjects, indomethacin was reported to produce modest but significant elevations in blood pressure (Staessen, Eagard, Lijnen and Amery, 1983). However, there are a number of reports that indomethacin does not alter blood pressure in normotensive animals and humans (Romero and Strong, 1977; Muirhead, Brooks and Brosius, 1976). Indomethacin, or similar drugs have been reported to aggravate hypertension in humans, Goldblatt hypertensive rats and rats with spontaneous hypertension. This is in contrast to other reports where there was no effect of indomethacin on rats or dogs with Goldblatt hypertension (McQueen and Bell, 1976; Yun, Kelly and Barrter, 1979). Seminal to the disparity in the results regarding the effects of cycloxygenase inhibitors on blood pressure is the effect these drugs have in reducing renin secretion and, consequently, plasma renin activity (Romero and

Strong, 1977). It is possible that the antihypertensive effect of reducing plasma renin activity with indomethacin may mask any tendency for blood pressure to rise because of reduced synthesis of vasodilator PGs.

1.8.2 Renal PG production

Renal cortical generation of PGs contributes to the regulation of renal blood flow, glomerular filtration rate and renin release. Infusion of AA into the renal artery of the rabbit is a strong stimulus for renin release and this release is blocked by indomethacin (Larsson, Weber and Anggard, 1974). Further experiments with AA showed that it can cause enhanced renal blood flow and sodium excretion in the dog, but in the rat AA can cause renin release independently of enhanced sodium and water excretion (Tannenbaum, Splawinski, Oates and Nies, 1975; Weber, Holzgreve, Stephan and Herbst, 1975). Using rabbit renal cortical slices it was found that AA, PGG_2 and PG endoperoxide analogues stimulate, while $PGF_{2\alpha}$ and indomethacin inhibit the release of renin; PGE_2 was without effect (Weber, Larsson, Anggard, Hamberg, Corey, Nicolaou and Samuelsson, 1976). PGI_2 is a potent stimulator of renin release from rabbit cortical slices (Whorton, Misono, Hollifield, Frolich, Inagami and Oates, 1977) or from the perfused kidney (Gerber, Branch, Nies, Gerkens, Shand, Hollifield and Oates, 1978; Bolger, Eisner, Ramwell, Slotkoff and Corey, 1978). In humans the augmented renin release after the diuretic frusemide is

inhibited by pre-treatment with indomethacin (Frolich, Hollifield, Dormois, Seyberth, Michelakis and Oates, 1976). In a non-filtering kidney, denervated and treated with β-adrenergic blocking agents, the increase in renal vein renin induced by reducing perfusion pressure is inhibited by indomethacin, indicating that PGs may contribute to the baroreceptor mechanism of renin release (Data, Crumb, Hollifield, Frolich and Nies, 1976). By involvement in these processes, renal PGs are part of the mechanisms controlling extracellular volume and blood pressure. The evidence to date suggests that in the intact kidney, AA and PGI_2 stimulate renin release, probably by a direct effect on the juxtaglomerular apparatus as well as an interaction with the baroreceptor, the β-adrenergic and macula densa receptor for renin release. Furthermore, the tubuloglomerular feed-back loop, involved in the control of sodium excretion is dependent on an intact PG system. The attenuation of the feed-back response to changes in loop of Henle flow rates after inhibition of PG synthesis can be restored by PGI_2 (Schnermann and Weber, 1980). There is also evidence that PG generation affects electrolyte secretion and vice-versa. Intra-renal infusion of PGE_2, PGD_2, PGI_2 and AA increase the urinary excretion of sodium, probably through effects on renal haemodynamics (for review see Dunn and Hood, 1977). Chronic salt depletion, on the other hand, increases renal PGE_2 production and release into the urine, whereas chronic high sodium intake suppresses PGE_2 formation. Thus, one of the known risk factors for hypertension, high sodium

intake may operate through a mechanism involving a reduction in PGE_2 formation.

1.8.3 Effects of PGs on neurotransmission

The hypotensive action of the PGs of the E series has been known for a long time, and it is now well established that PGE_2 inhibits the release of NA from many sympathetic nerves. This latter action has been shown in various species and in several different preparations (for review see Hedquist, 1977). The effect of PGI_2 on transmitter release from sympathetic nerves is contradictory. Hedquist (1979) found that PGI_2 had little or no effect on [^{3}H] NA release in response to nerve stimulation in rabbit kidneys, but Weitzell et al.(1978) reported inhibition of NA release by PGI_2 in rabbit pulmonary arteries. Armstrong et al.(1979) observed a similar effect with PGI_2 in rabbit mesenteric arteries, but not veins.

1.8.4 Correlations between PGs and pressure and flow in blood vessels

Using an alternative model of hypertension (i.e. portal hypertension), produced experimentally by partial ligation of the portal vein, it was shown that after 1 week of hypertension the portal veins of these rats released significantly greater amounts of PGI_2 (Hamilton, Rosa, Hutton, Chow, Dandona and Hobbs, 1981). In a further study it was found that during established

hypertension the release of PGI_2 was significantly greater than controls but as a collateral circulation developed with a consequent fall in portal pressure, PGI_2 release decreased and was significantly correlated with portal pressure (Hamilton, Phing, Hutton, Dandona and Hobbs, 1983). Using a different approach, Botha and Leary (1981) constricted the aorta of genetically hypertensive rats above the hepatic artery to produce a reduction in blood pressure distally. There was a significant reduction in tail pressure and segments of aorta produced decreased amounts of PGI_2 when compared to controls. The results of these studies suggest that PGI_2 release is enhanced when there is an increase in blood pressure and that when this influence is removed, PGI_2 release decreases again. Therefore, the increased production or release of PGI_2 from the blood vessels of genetically hypertensive rats may be due to a mechanical effect of an increased pulse pressure. Indeed, in the isolated, perfused aorta the use of a pulsatile pump appears to be a prerequisite for PGI_2 release (Quadt and Voss, 1982). Several studies have been carried out to determine the effect of mechanical pressure on PG production by blood vessels. Arteries have been shown to produce larger quantities of PGI_2 compared to veins (Skidgel and Printz, 1978). To test the hypothesis that PGI_2 production is related to blood pressure and flow, vein grafts were transplanted into the arterial circulation of dogs and rats. In the dog, although the venous grafts became arterialised from a structural point of view, there was no significant difference in PGI_2 production by grafts

compared with jugular veins (Eldor, Hoover, Pett, Gay, Alonso and Weksler, 1981). In the rat, however, PGI_2 production by vein grafts showed a progressive rise over a period of 42 days until levels were close to those of arterial controls (Petry, Burstein, Chang, Wortham, Sedor and Hunter, 1982).

1.8.5 Differences in PG production in hypertension

It has been proposed that a deficiency of production of vasodilator PGs in vascular beds (particularly the kidney) may lead to the increased peripheral vascular resistance characteristic of essential hypertension (Vane and McGiff, 1975). However, several groups have reported that the generation of PGI_2 and PGE_2-like substances from exogenous arachidonic acid is much greater in aortic rings and homogenates from spontaneously hypertensive rats (SHR) than in aorta from normotensive rats (Limas and Limas, 1977; Rioux, Quirion and Regoli, 1977; Pace-Asciak, Carrara, Rangaraj, Nicolaou, 1978). The release of a PGI_2-like substance was greater from aortic rings of stroke-prone and stroke-resistant SHR compared with normotensive controls (Okuma, Yamori, Ohta and Uchino, 1979), and was greater from aortic strips of the New Zealand genetically hypertensive (GH) strain compared to normotensive rats (Botha, Leary and Asmal, 1980).

Armstrong, Boura, Hamberg and Samuelsson (1976) found that GH rats were more sensitive to the hypotensive

action of the PG endoperoxides (PGG_2 and PGH_2) than normotensives but their sensitivity to PGE_2 was similar. Since the hypotensive action of the endoperoxides is probably due to PGI_2 this observation could be explained either by an increased conversion of endoperoxides to PGI_2, or by an enhancement of its vasodilator action in these GH rats. In a subsequent study (Dusting, Davies, Drysdale and Doyle, 1981) it was found that the vasodepressor effects of arachidonic acid were greater and more prolonged in SHR than in their Wistar-Kyoto controls, but that depressor responses to PGI_2 did not differ between strains. They concluded that the SHR had an enhanced ability to form vasodilator PGs from exogenous arachidonic acid. In a similar study it was concluded that rats with experimental renal hypertension (one kidney, one clip) had a greater ability to transform arachidonic acid into vasodilator PGs. However, SHR showed a greater depressor response to PGI_2 and a smaller depressor response to arachidonic acid compared with normotensive Sprague-Dawley rats, but not when compared with their usual control strain, the Wistar-Kyoto (Dusting, Di Nicolantonio, Drysdale and Doyle, 1981). This finding was confirmed by Lukascko, Messina and Kaley (1980) who found a larger depressor response to arachidonic acid in normotensive Wistar rats than in Wistar-Kyoto or SHR, suggesting a decreased capacity to utilise arachidonic acid for the synthesis of vasodilator PGs in both the SHR and Wistar-Kyoto strains of rat. An increased aortic generation of PGI_2 might result in a reduced platelet sensitivity to PGI_2, but Botha and Leary

(1981) found that platelets from SHR and normotensive rats were equally sensitive to the anti-aggregatory effect of PGI_2. The cyclooxygenase inhibitor ibuprofen, either given acutely or over 7 days had no significant effect on systolic blood pressure in SHR (Levy,1982). Using antibodies raised against 5,6-dihydro PGI_2 which cross-react with PGI_2, Pace-Asciak et al. (1980) found that although the vasodepressor effects of exogenous PGI_2 were blocked, the anti-5,6-dihydro PGI_2 antibodies had no effect on resting arterial blood pressure in normotensive rats or SHR. These latter results suggest that, contrary to the results described previously, the production of PGI_2 is not significantly increased in the blood vessels of genetically hypertensive rats, or that it is not contributing to the control of blood pressure in these animals.

Similar studies have been carried out to investigate PG production in experimental models of hypertension. The vasodepressor effects of PGI_2, nitroprusside and AA did not differ significantly between 2 kidney, 1 clip (2K, 1C) Goldblatt hypertensive rats compared to normotensives when measured as a percentage of resting blood pressure, in contrast to 1 kidney, 1 clip (1K, 1C), hypertensive rats which showed much greater depressor responses to AA (Dusting, Drysdale, Veroni and Doyle, 1983; Dusting, Dickens, Nicolantonio and Doyle, 1984). The ability of rings of arteries to produce PGs from [^{14}C] AA was examined in the initial (6 day) and chronic (6 week) phase of 2K, 1C hypertension; PGI_2 synthesis increased

2.4 times in the 6 day period of development of renovascular hypertension but there were no changes observed in PGI_2 synthesis in the 6 week period (Morera, Santoro, Roson and De la Riva, 1983).

Although there is a vast amount of literature dealing with PGI_2 and its actions on platelet aggregation and blood vessel tone, its proposed interaction with, and opposing effects to TXA_2, there is a paucity in the literature on the vascular production of two other major vascular PGs, $PGF_{2\alpha}$ and PGE_2. This is particularly true of the situation in the hypertensive state, which requires further study since an ab eration in the formation of any one PG relative to another may have profound effects on blood vessel tone and hence blood pressure. Moreover, it is generally basal PG production which is measured and the importance of an increased output in vasodilator PGs in response to vasoconstrictor stimuli, particularly in the hypertensive state, has not been established. Also, the majority of studies have been carried out using male rats although some differences in vascular PG production between male and female rats have been reported.

Similarly, in the aged animal, the increase in blood pressure observed with increasing age could involve changes in the profile of PG production by blood vessels, in addition to the structural changes in the blood vessel wall found with age.

The majority of studies investigating human vascular PG production have used tissue obtained from umbilical blood vessels which may not be representative of adult vascular tissue.

Consequently, experiments described in this thesis were undertaken to establish a more complete profile of basal and stimulated vascular PG production in normotensive and hypertensive, male and female rats. A study of vascular PG production was carried out in a large sample of male and female human subjects, undergoing coronary artery by-pass surgery. Since the analytical methods used are common to several sections, the next Section solely describes these analytical methods.

SECTION 2.0 Materials and General Methods

2.1.1 Introduction

The work presented in this thesis has involved the measurement of PGs in aorta, vena cava and mesenteric arterial bed of male and female rats, and in aorta and saphenous vein of human subjects who were undergoing coronary artery bypass surgery.

PGs were extracted from incubates of homogenised vascular tissue or from perfusates of isolated vascular preparations into organic solvents and were measured by radioimmunoassay (RIA). The PGs measured by RIA in the tissue homogenates were also identified by combined gas chromatography and mass spectrometry (GC-MS). The procedures described here for extracting, measuring and identifying the PGs are common to each section.

2.1.2 Materials

Solvents

Acetic acid, glacial (Analar)	B.D.H. Chemicals Ltd., U.K.
Absolute ethanol	J. Boroughs Ltd., U.K.
Chloroform	B.D.H. Chemicals Ltd., U.K.
2- Ethoxyethanol (Analar)	B.D.H. Chemicals Ltd., U.K.
Ethyl Acetate (reagent grade)	B.D.H. Chemicals Ltd., U.K.
Methanol (Analar)	Fisons, U.K.
Toluene (Analar)	Fisons, U.K.

Ethyl acetate was redistilled prior to use.

Radioactive Compounds

5,8,9,11,12,14,15(n)-^{3}H) 6-keto-prostaglandin $F_{1\alpha}$
(sp.act 150 Ci/mmol)

5,6,8,11,12,14,15(n)-^{3}H) Prostaglandin $F_{2\alpha}$
(sp.act. 160 Ci/mmol)

5,6,8,11,12,14,15(n)-^{3}H) Prostaglandin E_2
(sp. act. 160 Ci/mmol)

All from Amersham International Ltd., U.K.

^{3}H $PGF_{2\alpha}$ and ^{3}H PGE_2 were stored at 5 μCi/ml in methanol, at -20°C and ^{3}H 6-keto-$PGF_{1\alpha}$ was stored at 5 μCi/ml in acetonitrile: water, 9:1 v/v at -20°C.

2.1.3 Other Chemicals

Authentic PGs and their metabolites were the gift of Dr. J. Pike, Upjohn Company, Kalamazoo, USA.

Angiotensin II	Sigma Chemical Company, UK
Arachidonic acid	Sigma Chemical Company, UK
Noradrenaline	Sigma Chemical Company, UK
Oestradiol benzoate	Sigma Chemical Company, UK
Progesterone	Sigma Chemical Company, UK

2.2.1 Composition of Kreb s solution

2g	glucose
2.1g	sodium hydrogen carbonate
6.9g	sodium chloride
1.4 ml	10% magnesium sulphate
1.6 ml	10% potassium dihydrogen orthophosphate
3.5 ml	10% potassium chloride
2.5 ml	1M calcium chloride

Kreb s solution was made up daily in 1l quantities.

All reagents were supplied by B.D.H. Chemicals Ltd., U.K.

2.2.2 Composition of McEwen's solution

2.1g	glucose
4.2g	sucrose
7.6g	sodium chloride
2.1g	sodium hydrogen carbonate
4.2ml	10% potassium chloride
1.4ml	10% potassium dihydrogen orthophosphate
2.4ml	1M calcium chloride

McEwen's solution was made up daily in 1l quantities.

All reagents were supplied by B.D.H Chemicals Ltd., UK

2.3.1 Chemicals and solutions for RIA

Tris (hydroxymethyl)-methylamine	B.D.H. Chemicals Ltd., UK
di-sodium hydrogen orthophosphate	B.D.H. Chemicals Ltd., UK
sodium dihydrogen orthophosphate	B.D.H. Chemicals Ltd., UK
sodium azide	Hopkin and Williams Ltd., UK
gelatine	B.D.H. Chemicals Ltd., UK
P.P.O (2,5-diphenyloxazole)	Fisons, UK

2.3.2 Diluents for RIA

6-keto-$PGF_{1\alpha}$ assay

0.05 M Tris buffer pH 6.8

0.1 g/l Sodium azide

1.0 g/l Gelatine

$PGF_{2\alpha}$ assay

0.05 M Tris buffer pH 8.0

0.1 g/l Sodium azide

1.0 g/l Gelatine

PGE_2 assay

0.05 M Phosphate buffer pH 7.5

0.1 g/l Sodium azide

0.1 g/l Gelatine

Scintillation Fluid

1.5 l Toluene

0.9 l 2-Ethoxyethanol

10.5 g P.P.O

Donkey anti-rabbit serum (DARS) - Wellcome Reagents Ltd. and Scottish Antibody Production Unit, UK. Normal rabbit serum (NRS) was obtained from non-immunised male New Zealand white rabbits by the method of Dighe, Emslie, Henderson and Simon (1975).

2.4 Tissue homogenisation and solvent extraction of PGs

Rats were killed by stunning and incising the neck. The aorta was removed, washed to remove trapped blood, blotted dry and weighed. The tissue was then chopped finely with scissors and homogenised in a Fisons glass homogeniser with 5 ml Krebs solution. The homogeniser was washed with 5 ml Krebs solution and the homogenate and washings were added to a 25 ml conical flask. The homogenate was incubated with 2 μg/ml arachidonic acid for 60 min at 37°C, in a Grant water-bath, and was gassed with 95% O_2, 5% CO_2.

PGs were extracted from homogenates using the method of Poyser (1972). The pH of the homogenate was lowered to pH4 by the dropwise addition of 1N HCl. The homogenate was then extracted twice with two volumes of ethyl acetate. The ethyl acetate fractions were combined and evaporated to dryness under reduced pressure at 45°C on a rotary evaporator. The residue was redissolved in 4ml of

ethyl acetate and was stored at -20°C until assayed.

It is well established that extracting PGs by this method from Krebs solution gives a high recovery for 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2. The recoveries of the appropriate radioactive PGs at pH4 were found to be 82 +/- 2% for 6-keto-$PGF_{1\alpha}$ (Swan and Poyser, 1983), 94 +/- 3% for $PGF_{2\alpha}$ and 92 +/- 5% for PGE_2 (Poyser and Scott, 1980). PG concentrations measured in tissue homogenates and perfusates have not been corrected for procedural losses.

2.5 Measurement of PGs by radioimmunoassay

2.5.1 Assay protocol

The procedure for setting up a radioimmunoassay is essentially the same for 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2. Therefore, the general procedure for the assay will be described followed by the specific details of solutions, antisera and crossreactivities for each prostaglandin assay.

6-keto-$PGF_{1\alpha}$ and PGE_2 antisera were raised in rabbits after immunisation with a 6-keto-$PGF_{1\alpha}$-thyroglobulin conjugate (Dighe, Jones and Poyser, 1978) and a PGE_2-thyroglobulin conjugate (Dighe, Smith, Ungar and Whelpdale, 1978). $PGF_{2\alpha}$ antiserum was raised in rabbits using a $PGF_{2\alpha}$-bovine serum albumin conjugate (Dighe, Emslie, Henderson, Rutherford and Simon, 1975).

The mass of [^{3}H] prostaglandin ([^{3}H] PG) used was chosen to give approximately 20,000 counts in a 50 ul aliquot, counted over a period of 4 min. The solvent containing the [^{3}H] PG was evaporated off under a stream of air and the [^{3}H] PG was redissolved in diluent.

The dilution of antiserum chosen was that which bound 60% of the [^{3}H] PG in the absence of any non-radioactive PG. A stock solution of antiserum in diluent, of 1:100 was prepared and this was used to prepare the appropriate final dilution.

Standard solutions were made up in diluent from a stock solution of 1 μg/ml in methanol. An appropriate quantity of the PG in methanol was taken and the methanol was evaporated off under a stream of air. The PG was redissolved in diluent to give a PG concentration of 10.24 ng/ml. Nine serial dilutions were made from this highest concentration. Disposable polypropylene tubes containing 0.5 ml of the standard PG solutions were set up in triplicate as detailed in Table 2.5.1. Tubes were also set up in quadruplicate for counting standards, zero standards and non-specific binding standards.

Table 2.5.1

TUBE CODE	ng PG / TUBE	COMMENTS
MB	0	1 Machine Background - no additions
CS	0	4 counting standards -50 µl [^{3}H] PG only
No. 1-4	5ng	Non specific binding standards
5-8	0	Zero standards
9-11	2.56	PG standard curve
12-14	1.28	"
15-17	0.64	"
18-20	0.32	"
21-23	0.16	"
24-26	0.08	"
27-29	0.04	"
30-32	0.02	"
33-35	0.01	"

The counting standards give an estimate of the average number of radioactive counts added to each tube. The zero standards indicate the degree of binding of the [^{3}H] PG to the antiserum in the absence of non-radioactive PG. The non-specific binding standards, which contain sufficient non-radioactive PG to completely saturate the specific sites of the antiserum, indicate the

non-specific binding of the [^{3}H] PG to other 'components' of the assay. Non specific binding was always less than 6%.

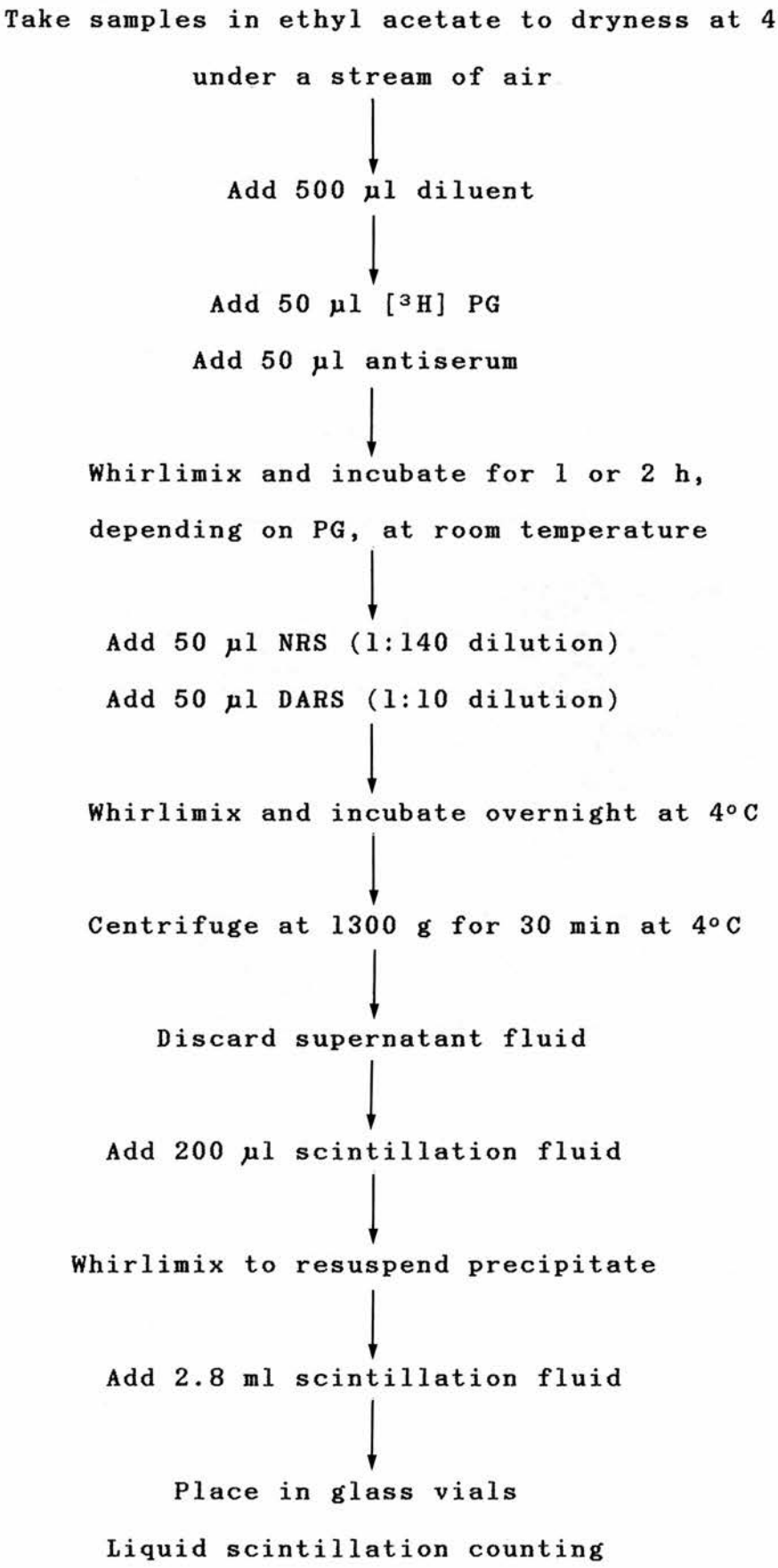
The assay protocol is detailed in Fig. 2.5.1. Extracted samples in ethyl acetate were dispensed into disposable tubes in two different volumes, and in duplicate, by fixed volume Eppendorff pipette with disposable tips. The use of different volumes was to ascertain if parallelism existed between the volumes. Samples were taken to dryness at 45°C under a stream of air, and were redissolved in 500 µl of diluent. The samples and standard PG solutions were then treated in the same way. 50 µl of antiserum and 50 µl of [^{3}H] PG were added to each tube, the tubes were whirlimixed and left to incubate at room temperature, for 1 hour if the assay was for $PGF_{2\alpha}$ and 2 hours if the assay was for 6-keto-$PGF_{1\alpha}$ or PGF2. After incubation, 50 µl of normal rabbit serum (NRS) and 50 µl donkey anti-rabbit serum (DARS) were added to all the assay tubes which were then whirlimixed. The tubes were left to incubate overnight at 4°C.

NRS was added after incubation to ensure that at higher dilutions of the antiserum there was sufficient gamma-globulin present for adequate precipitation. DARS was added to separate free from antibody-bound PG.

The next morning the tubes were centrifuged at 1300 g for 30 min at 4°C. The supernatant fluid was poured off and was discarded. 200 µl of scintillation fluid were added

to each tube, the tubes were whirlimixed to resuspend the precipitate and a further 2.8 ml of scintillation fluid were added to each tube. The tubes were put into glass vials and were placed in a liquid scintillation counter.

Fig. 2.5.1


Take samples in ethyl acetate to dryness at 45°C
under a stream of air
Add 500 µl diluent
Add 50 µl [³H] PG
Add 50 µl antiserum
Whirlimix and incubate for 1 or 2 h,
depending on PG, at room temperature
Add 50 µl NRS (1:140 dilution)
Add 50 µl DARS (1:10 dilution)
Whirlimix and incubate overnight at 4°C
Centrifuge at 1300 g for 30 min at 4°C
Discard supernatant fluid
Add 200 µl scintillation fluid
Whirlimix to resuspend precipitate
Add 2.8 ml scintillation fluid
Place in glass vials
Liquid scintillation counting

The vials were counted for 4 min in a Nuclear Chicago Liquid Scintillation counter and the results recorded on a paper punch tape. The tape was fed into a PDP-8 computer or, latterly, a Commodore 4032 computer, both programmed to calculate the percentage binding of the [^{3}H] PG. This was calculated using the formula.

$$\% \text{ bound} = \frac{(\text{sample counts} - \text{background counts})}{(\text{average counting standard counts} - \text{background counts})} \times 100$$

The computer applied a logistic curve fitting formula to the observed values of percentage [^{3}H] PG bound in the standard curve and calculated the coordinates for the curve of best fit. The two sets of values obtained, (i.e. observed values and calculated values) were compared, and if the variation between them was less than 5% the curve was accepted. If the gradient of the standard curve was less than 8%, as measured over a doubling of the concentration of PG per tube, the assay was repeated. Values for the 'extracted PG' which did not lie on the linear part of the curve were reassayed using different dilutions of the sample.

The intra assay coefficient of variation was calculated from the formula:

$$\text{Intra assay coefficient of variation} = \frac{\sum \left[\frac{\text{standard deviation of sample duplicate}}{\text{mean value of sample duplicate}} \right] \times 100}{\text{total number of duplicates}}$$

Two tubes containing a known amount of the appropriate PG were set up in duplicate at the end of each assay and these results were used to calculate the interassay coefficient of variation, using the formula:

$$\text{Inter assay coefficient of variation} = \frac{\text{standard deviation of standard}}{\text{mean of standard}} \times 100$$

Crossreactivities for each of the PG antisera have been determined by Miss Isa Ramsay. A standard curve for the PG was set up in parallel with standard curves for different prostaglandins and their metabolites. The concentration of PG or PG metabolite which produced a 10% fall in binding from the zero standard was obtained and the percentage cross-reactivity was calculated using the following formula.

$$\%\ \text{cross reactivity} = \frac{\text{concentration of the PG giving a 10\% fall in zero binding}}{\text{concentration of PG or PG metabolite giving a 10\% fall in zero binding}} \times 100$$

2.5.2 6-keto-$PGF_{1\alpha}$ Radioimmunoassay

A standard curve for 6-keto-$PGF_{1\alpha}$ was set up as in Table 2.5.1. The assay protocol detailed in Fig. 2.5.1 was followed.

Reagents

Tris diluent pH 6.8

^{3}H 6-keto-$PGF_{1\alpha}$ (sp. act. 150 Ci/mmol)

6-keto-$PGF_{1\alpha}$ antiserum (rabbit 1, 6th bleed)

1:1500 dilution

NRS 1:140 dilution

DARS 1:10 dilution

Incubation time at room temperature -2h.

Results

Fig. 2.5.2 shows the standard curve for the 6-keto-$PGF_{1\alpha}$ radioimmunoassay drawn from the results of 6 consecutive assays. (Mean +/- s.e.m).

Detection limit 40 pg

Intra assay coefficient of variation 9.5%

Inter assay coefficient of variation 6.5%

Table 2.5.2 shows the cross reactivities of the 6-keto-$PGF_{1\alpha}$ antiserum, rabbit 1, 6th bleed with other prostanoids as determined by Miss I. Ramsay. Cross reactivity was measured at 30% binding of tracer.

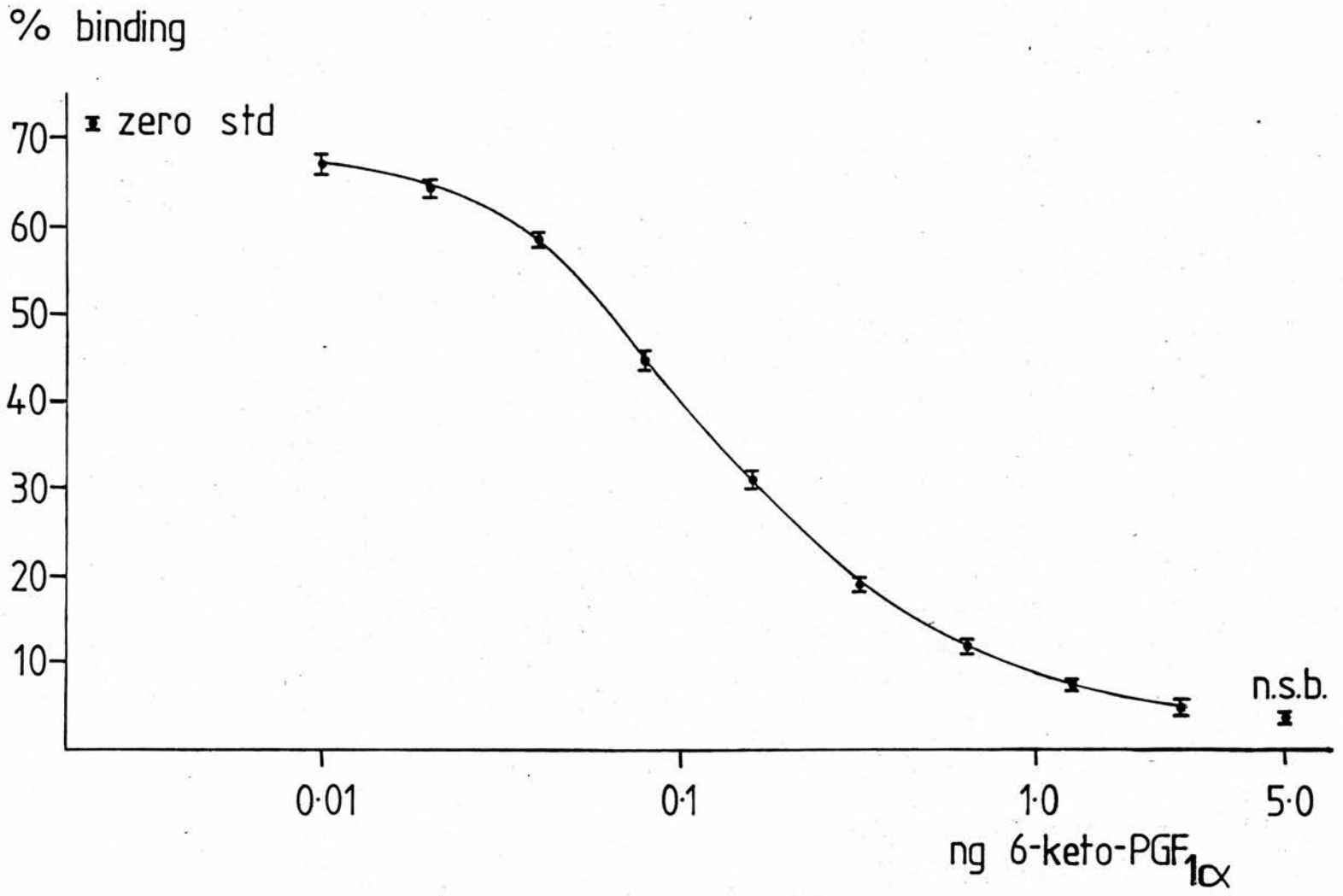


Fig. 2.5.2. Standard curve for 6-keto-$PGF_{1\alpha}$ radioimmunoassay; mean ± s.e.m. of 6 consecutive assays. n.s.b = non-specific binding.

Table 2.5.2

Prostanoid	% cross reactivity at 30% binding of tracer
6-keto-$PGF_{1\alpha}$	100
$PGF_{1\alpha}$	0.43
PGE_2	4.2
PGE_1	1.1
PGA_2	0.065
PGB_2	0.03
$PGF_{2\alpha}$	0.01
PGD_2	0.01
15-keto-$PGF_{2\alpha}$	0.04
13,14-dihydro,15-keto-$PGF_{2\alpha}$	0.065
15-keto-PGE_2	0.08
13,14-dihydro,15-keto-PGE_2	0.09
TXB_2	0.01

As there was no significant cross reactivity found with any of the prostanoids tested, this 6-keto-$PGF_{1\alpha}$ antibody was considered suitable for the measurement of 6-keto-$PGF_{1\alpha}$.

2.5.3 $PGF_{2\alpha}$ Radioimmunoassay

A standard curve for $PGF_{2\alpha}$ was set up as in Table 2.5.1. The assay protocol described in Fig. 2.5.1 was followed.

Reagents

Tris diluent, pH 8.0

^{3}H $PGF_{2\alpha}$ (sp. act. 160 Ci/mmol)

$PGF_{2\alpha}$ antiserum (rabbit 6, 4th bleed)

1:1600 dilution

NRS 1:140 dilution

DARS 1:10

Incubation time at room temperature, 1h.

Results

Fig. 2.5.3 shows the standard curve for the $PGF_{2\alpha}$ radioimmunoassay drawn from the results of 6 consecutive assays (mean +/- s.e.m.).

Detection limit 30 pg

Intra assay coefficient of variation 9.4%

Inter assay coefficient of variation 7.9%

Table 2.5.3 shows the cross reactivities of the $PGF_{2\alpha}$ antiserum, rabbit 6, 4th bleed with other prostanoids, as determined by Miss I. Ramsay.

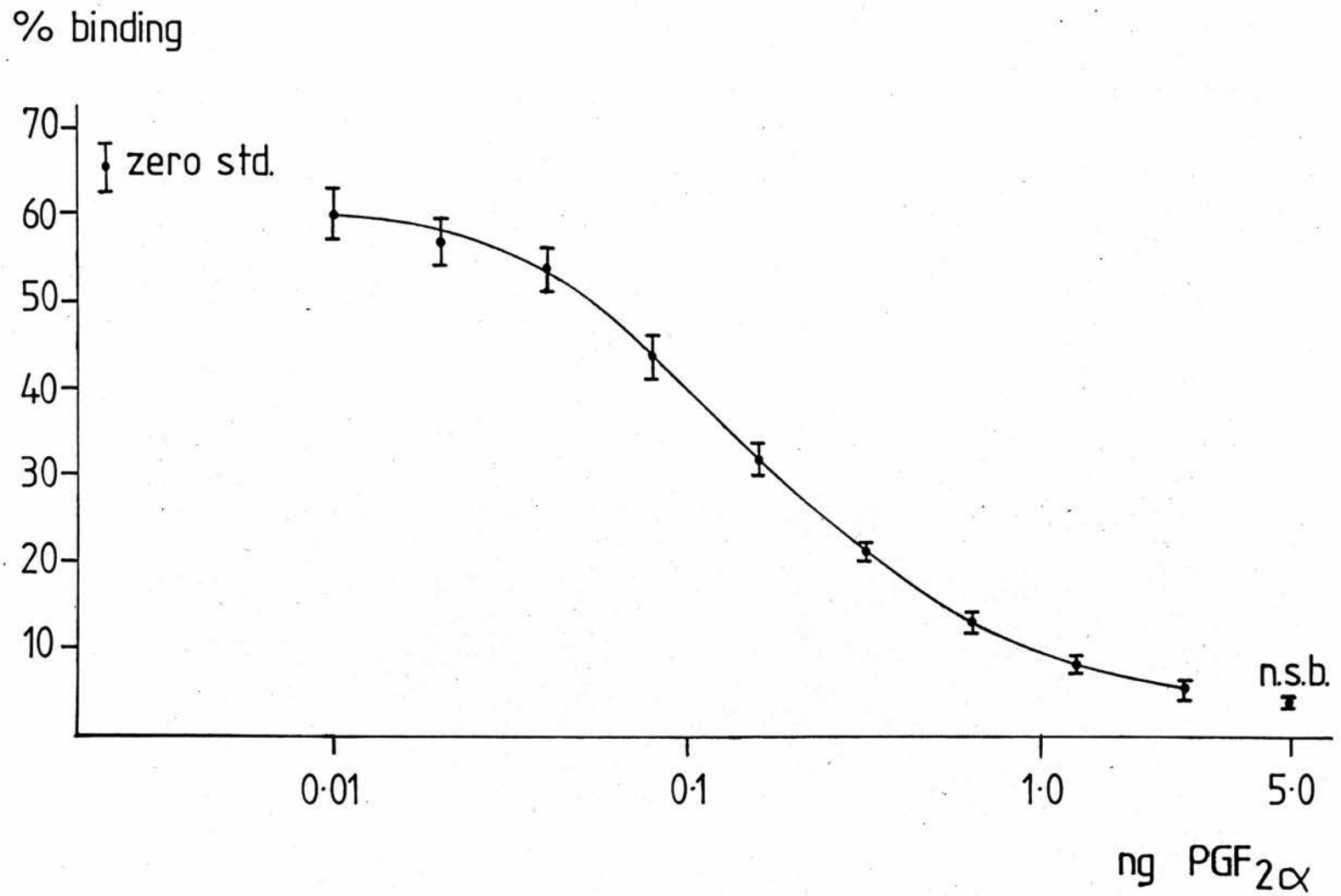


Fig. 2.5.3. Standard curve for $PGF_{2\alpha}$ radioimmunoassay; mean ± s.e.m. of 6 consecutive assays. n.s.b. = non-specific binding.

Table 2.5.3

Prostanoid	% cross reactivity at 30% binding of tracer
$PGF_{2\alpha}$	100
$PGF_{1\alpha}$	100
PGE_2	0.56
PGE_1	0.60
PGA_2	0.07
PGB_2	0.01
PGD_2	0.30
15-keto-$PGF_{2\alpha}$	0.10
13,14-dihydro, 15-keto-$PGF_{2\alpha}$	0.02
6-keto-$PGF_{1\alpha}$	1.1
TXB_2	0.1

The $PGF_{2\alpha}$ antiserum cross reacts 100% with $PGF_{1\alpha}$. Samples were therefore subjected to further analysis by GC-MS to determine if significant quantities of $PGF_{1\alpha}$ had been produced.

2.5.4 PGE_2 Radioimmunoassay

A standard curve for PGE_2 was set up as described in Table 2.5.1. The assay protocol in Fig. 2.5.1 was followed.

Reagents

Phosphate diluent, pH 7.5

^{3}H PGE_2 (sp. act. 160 Ci/mmol)

PGE_2 antiserum (rabbits 5, 7th bleed)

1:400 dilution

NRS 1:140 dilution

DARS 1:10 dilution

Incubation time at room temperature- 2 h

Results

Fig. 2.5.4 shows the standard curve for the PGE_2 radioimmunoassay drawn from the results of 6 consecutive assays (mean +/- s.e.m).

Detection limit 40 pg

Intra-assay coefficient of variation 11.8%

Inter-assay coefficient of variation 12.2%

Table 2.5.4 shows the cross reactivities of the PGE_2 antiserum, rabbit 5, 7th bleed with other prostanoids as measured by Miss I. Ramsay.

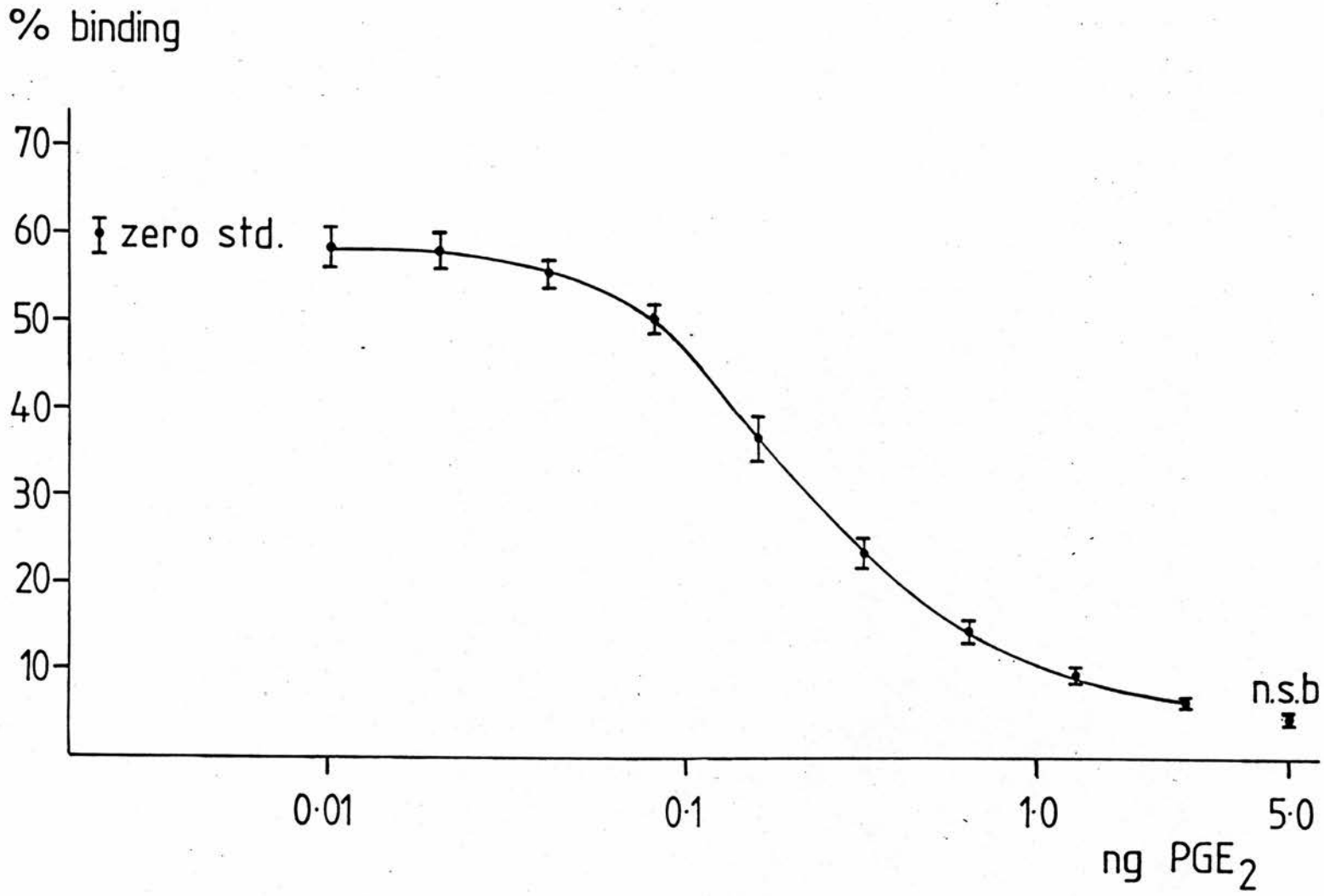


Fig. 2.5.4. Standard curve for PGE_2 radioimmunoassay; mean ± s.e.m. of 6 consecutive assays. n.s.b = non-specific binding.

Table 2.5.4

Prostanoid	% cross reactivity at 50% binding of tracer
PGE_2	100
PGE_1	94
PGA_2	13.6
PGB_2	72.7
$PGF_{2\alpha}$	1.5
PGD_2	0.4
15-keto-PGE_2	0.2
13,14-dihydro, 15-keto PGE_2	0.1
15-keto-$PGF_{2\alpha}$	1.1
13,14-dihydro, 15-keto $PGF_{2\alpha}$	0
6-keto-$PGF_{1\alpha}$	1.1
TXB_2	0.2

The PGE_2 antibody showed high cross reactivity with PGE_1 and PGB_2, so the samples were subjected to further analysis by GC-MS to determine if they contained significant quantities of PGE_1, PGA_2 and PGB_2 which might interfere with the measurement of PGE_2 by this radioimmunoassay.

2.6 Column Chromatography and Gas Chromatography Mass Spectrometry

2.6.1 Introduction

Radioimmunoassay provides a sensitive method for measuring picogram (pg) quantities of PGs in biological samples, routinely and on a large scale. However, this technique does not provide an unequivocal identification of the compounds measured. Extraction of the PGs from the biological fluid decreases the possibility of other compounds in the sample cross-reacting non-specifically with the antibody, and cross-reactivity studies with structurally related compounds give a further indication of the specificity of the assay for a particular PG. However, such studies are necessarily confined to compounds identified previously and available in a pure form.

The cross-reactivities of the different antisera have been detailed in the previous section and it was found that the $PGF_{2\alpha}$ anti-serum cross-reacts significantly with $PGF_{1\alpha}$, and that the PGE_2 anti-serum cross-reacts significantly with PGA_2, PGB_2 and PGE_1. Samples from aorta and vein which had been assayed for PGs by RIA were therefore analysed further by combined gas chromatography and mass spectrometry (GC-MS) to identify positively 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 in the samples and also to determine if these samples contained significant quantities of $PGF_{1\alpha}$, PGA_2, PGB_2 and PGE_1. There were insufficient samples left from vena cava, femoral artery

or mesenteric artery for analysis by GC-MS. Before GC-MS analysis PGs were separated from tissue extracts by column chromatography.

2.6.2 Purification of tissue extracts by column chromatography

Columns (12 cm x 1 cm) containing 4 g of silicic acid (Unisil, Williamsport, Pennsylvania, USA, 100-200 mesh) were prepared and were washed with 100 ml of diethyl ether.

The ethyl acetate extracts of one tissue type were combined to give pooled samples (i.e. aorta samples from rats, and aorta and saphenous vein from humans). Each unit pooled sample was evaporated to dryness under reduced pressure at 45°C was redissolved in 30 ml of 67% ethanol, was shaken twice with petroleum ether (b.pt 40-60°C) and the ethanol fraction was evaporated to dryness again. Each sample was then dissolved in 0.5 ml diethyl ether and applied dropwise to the top of a column. The columns were eluted by 100 ml diethyl ether followed by 100 ml of 80% diethyl ether and 20% methanol. The first solvent elutes neutral lipids, including fatty acids, and would have therefore removed any unconverted arachidonic acid. The second solvent mixture elutes PGs, and this fraction from each column was evaporated to dryness and was redissolved in 1 ml ethyl acetate.

2.6.3 Gas chromatography - Mass spectrometry (GC-MS)

GC-MS analysis was carried out on a VG-Micromass 7070 F

double focussing gas chromatogram-mass spectrometer. The column (3m x 4mm) was packed with 3% OV 1 on supelcoport 100-120 mesh (Supelco Inc., Bellefonte, USA). The conditions for operation of the mass spectrometer were:

Column temperature	260°C
Ion source temperature	250°C
Electron energy	70eV
Accelerating voltage	4kV
Signal amplification	2 x 10-6 amps

Full mass spectra were recorded on photographic paper by a light beam oscillograph (S.E. Labs (EMI) Ltd., Feltham, UK). A mixture of straight chain saturated fatty acid methyl esters with 16, 18, 20, 22 and 24 carbon atoms was used to calibrate the GC column. By plotting the retention times of each fatty acid on a logarithmic scale, the observed retention times of the standard PGs could be converted to carbon values. The size and shape of the GC peak corresponding to each fatty acid also indicates the efficiency of the GC column.

2.6.4 Derivatisation of PGs for GC-MS

The presence of carboxyl and hydroxyl groups in the free PGs make these compounds too polar to be run directly on a gas chromatograph. Different derivatives have therefore been used to reduce the polarity of these functional groups. Carboxylic acid groups on the PGs were protected by forming methyl ester derivatives, free

hydroxyl groups were protected by trimethylsilyl ether derivatives, and free ketone groups were protected by conversion to butyl oxime derivatives.

2.6.4.1 Method

Two 0.5 ml aliquot portions of each sample were evaporated to dryness in 1 ml eppendorff tubes at 45°C under a stream of air. Authentic PG standards in methanol were dispensed into 1 ml eppendorff tubes and were also evaporated to dryness.

2.6.4.2 Methyl ester formation

To all the tubes was added 0.5 ml diazomethane solution (diazomethane in 9 parts diethyl ether, 1 part methanol), and the contents of the tubes were allowed to react for 10 min. The solvent was evaporated off at 45°C under a stream of air. Drying was completed by vacuum dessication for 15 min.

2.6.4.3 n-Butyloxime formation

Three drops of 5 mg/ml butyl hydroxylamine hydrochloride in dry pyridine were added to one tube from each sample and to tubes containing PGE_1, PGE_2, PGD_2, 6-keto-$PGF_{1\alpha}$ and TXB_2. The tubes were stoppered and the contents allowed to react overnight at room temperature. The next morning the tubes were heated at 60°C under a stream of air to complete the reaction and to evaporate off the

pyridine.

2.6.4.4 Trimethylsilyl ether formation

Bis(trimethylsilyl)-trifluoroacetamide (BSTFA; 30µl) was added to each standard and 15 ul BSTFA was added to each sample. After dosing the tubes and whirlimixing the contents, the tubes were heated for 15 min at 60°C.

Each standard PG was injected into the gas chromatograph in 10 ul BSTFA. Their retention times on the GC were recorded and a full mass spectrum was taken. Each sample was then injected into the instrument and a mass spectrum taken at the appropriate retention time. The mass spectra obtained from the samples were then compared with those from the standard PGs. The limit of detection of the mass spectrum is approximately 50 ng. However, to obtain a full mass spectrum about 300 ng of the extracted PG is required and in some instances there may not have been sufficient PG present in the pooled sample to be detected on the mass spectrum. Table 2.6.1 shows the characteristic ions used to determine the presence of PGs and TXB_2 in biological samples.

Table 2.6.1

Characteristic ions of methyl trimethylsilyl ether (Me, TMS) and methyl trimethylsilyl butoxime (Me, Buo, TMS) derivatives of PG and TXB_2 standards (m = mass of ion, e is the ionic charge of ion = 1).

Compound	Derivative	Characteristic ions (m/e value)
PGA_2	Me, TMS	420, 349, 190
PGB_2	Me, TMS	420, 349, 173
$PGF_{1\alpha}$	Me, TMS	586, 513, 497, 425, 406
$PGF_{2\alpha}$	Me, TMS	584, 513, 494, 423, 404
6-keto-$PGF_{1\alpha}$	Me, Buo, TMS	671, 656, 600, 598, 510,
1st isomer		508, 491
PGD_2	Me, Buo, TMS	581, 510, 508, 420, 418, 295
2nd isomer		
PGE_1	Me, Buo, TMS	581, 568, 510, 512, 297
1st isomer		
PGE_2	Me, Buo, TMS	581, 566, 510, 508, 295, 225
2nd isomer		
TXB_2	Me, Buo, TMS	301, 211

SECTION 3.0

Measurement of PG production in homogenates of aorta, vena cava mesenteric artery and femoral artery of male rats.

3.1.1 Introduction

Since the discovery of the ability of rabbit aortic microsomes to synthesise prostacyclin reported by Moncada et al. (1976), prostacyclin has been assumed to be the major product of arachidonic acid metabolism in all vascular tissues. Other PGs identified in vascular tissues have been suggested to have relatively unimportant roles.

However, there is some evidence that PGI_2 is not the only PG produced by vascular tissue. PGE_2, a potent vasodilator and $PGF_{2\alpha}$, a vasoconstrictor have been shown to be produced by bovine aorta and bovine mesenteric arteries and veins (Terragno et al., 1975), and by pulmonary artery and mesenteric artery of foetal and maternal origin (Terragno et al., 1978). TXA_2 synthesis by vascular tissue has also been reported. TXA_2 (measured as TXB_2) is produced by the rabbit pulmonary artery (Salzman et al., 1980), by vascular endothelial cells in culture (Ingerman-Wojenski et al., 1981; Goldsmith and Needleman, 1982), and by slices of human umbilical artery, piglet aorta and vena cava (Siess et al., 1981). The amounts of TXA_2 represented by the TXB_2 measured in these studies was very small, trace amounts

in some cases, and their role in the vascular wall is not known at present. It should also be considered that in some instances, the production of TXA_2 by tissues is due to the presence of platelets. TXB_2 has not been measured in the experiments described here, because of the difficulty in completely removing platelet contamination, and because there was insufficient sample to measure 3 PGs and TXB_2.

The aorta, because of its size and accessibility, is the vessel most often used in studies of vascular PG production. However, the aorta is not a major resistance vessel and may not be typical of all blood vessels in its production of PGs; therefore three other blood vessels, the vena cava, the mesenteric artery and the femoral artery have also been investigated in this study. The aim of the experiments described in this section was to characterise the overall profile of PG production by these blood vessels.

Gender differences in PGI_2 production have been reported, with males found to release more 6-keto-$PGF_{1\alpha}$ from aortic rings than females (Pomerantz et al. 1980). Furthermore, male lungs convert labelled or unlabelled arachidonate into 6-keto-$PGF_{1\alpha}$ more readily than female lungs, (Maggi et al., 1980). Therefore, males and females will be treated separately and the results from the 2 groups compared and discussed in Section 3.4.5.

3.1.2 Methods

Male albino Wistar rats, aged between 2 and 3 months and weighing 200-250 g, were used. The animals were kept under controlled lighting conditions and allowed free access to food and water.

Six animals were killed by stunning and incising the neck between 10.00 h and 12.00 h. The aorta and vena cava from each rat were dissected free from surrounding tissue, washed in Krebs solution, blotted dry and weighed. The aorta and vena cava were homogenized separately in 5 ml Krebs solution and the homogenates were each added to a 50 ml conical flask. The homogeniser was washed with 5 ml Krebs solution and the washings were added to the flasks. The homogenates were incubated for 60 min with 2 μg/ml arachidonic acid. After incubation the pH of each homogenate was taken to pH4 with 1N HCl and the PGs were extracted with ethyl acetate as described in Section 2.4. Samples were stored in 4 ml ethyl acetate at -20°C until they were assayed by RIA, for their 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 content as described in Section 2.5.

Another identical group of 6 male rats was used to investigate PG production by the femoral artery and mesenteric artery. The animals were killed and the femoral and mesenteric arteries were removed and weighed. These vessels were homogenised and incubated, and the PGs were extracted and assayed as described above.

3.1.3 Results

Figure 3.1.1 shows the mean (+/- s.e.m, n = 6) amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 formed from exogenous arachidonic acid in homogenates from aorta, vena cava, femoral artery and mesenteric artery. 6-Keto-$PGF_{1\alpha}$ was the major PG produced by homogenates of aorta with lesser amounts of $PGF_{2\alpha}$ and PGE_2. Homogenates of vena cava and femoral artery produced similar amounts of the 3 PGs. The mesenteric artery produced similar amounts of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ with less PGE_2. Femoral artery PG production per mg of tissue was the lowest of the 4 tissues. 6-Keto-$PGF_{1\alpha}$ production by aorta was much higher (3 to 8 fold) than the other vascular tissues, although aortic $PGF_{2\alpha}$ and PGE_2 production were comparable to the other tissues.

3.1.4 Conclusions

These experiments established that although the major PG production by homogenates of aorta was PGI_2 (measured as 6-keto-$PGF_{1\alpha}$), there were also considerable amounts of $PGF_{2\alpha}$ and PGE_2 produced. Surprisingly, in the other tissues, the vena cava, mesenteric artery and femoral artery all 3 PGs were produced to a similar degree. Thus, only in the aorta was this large disparity in 6-keto-$PGF_{1\alpha}$ production observed.

3.1.5 GC-MS Results

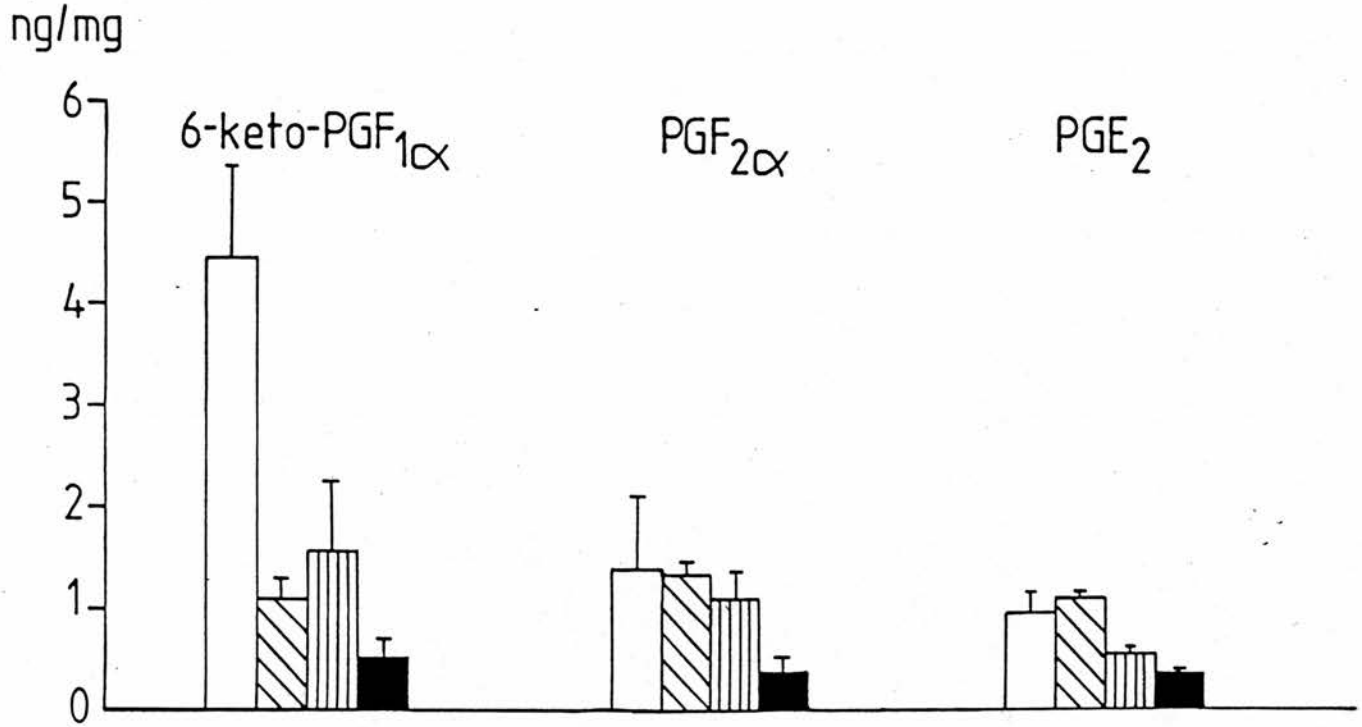


Fig. 3.1.1. Mean (+/- sem, n=6) amounts of prostaglandins (PGs) Synthesised by homogenates of aorta, ☐ vena cava, ▨ mesenteric artery, ▥ and femoral artery, ■ of male rats.

The cross reactivity studies given in Section 2.5 showed that the $PGF_{2\alpha}$ antiserum cross-reacted significantly with $PGF_{1\alpha}$ and that the PGE_2 antiserum cross-reacted significantly with PGA_2, PGB_2 and PGE_1. A pooled sample from the aortic homogenates was therefore analysed further by GC-MS to identify positively the presence of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 and to investigate if there was sufficient PGA_2, PGB_2, PGE_1 or $PGF_{1\alpha}$ present to interfere with the RIA. Unfortunately, there was insufficient sample from the other tissues for analysis by GC-MS. The procedure for the GC-MS analysis is given in Section 2.6.

The presence of 6-keto-$PGF_{1\alpha}$ was positively identified in the pooled sample from aorta. The presence of PGE_2 was difficult to determine unequivocally but the detection of small peaks at the appropriate carbon value did seem to indicate that there was PGE_2 present. Although no $PGF_{2\alpha}$ could be found this was probably because even in the pooled sample there was insufficient $PGF_{2\alpha}$ to be detected in a full mass spectrum. Similarly, the peaks characteristic of PGA_2, PGB_2, PGE_1 and $PGF_{1\alpha}$ could not be detected, so it cannot be stated whether these compounds were absent from or present in the samples. However, since arachidonic acid was present in the incubations in excess, it is unlikely that substantial quantities of PGE_1 and $PGF_{1\alpha}$ were formed. Furthermore, an enzyme converting PGE_2 into PGA_2 (and hence to PGB_2) in tissues has not been conclusively demonstrated, so it is again unlikely that substantial quantities of PGA_2 and PGB_2

were present. Consequently, the antibodies were probably predominantly measuring the PG to which they had been raised.

SECTION 3.2

Measurement of aortic PG levels

3.2.1 Introduction

The PG content of extracts of aortic homogenates after a 60 min incubation period has thus far been described as PG 'production'. Before 'production' can be equated with 'synthesis', it is necessary to determine that (i) prior to tissue homogenisation and incubation; the levels of PGs in the aortic tissue are low, and (ii) that metabolism of PGs by the homogenate during incubation is also low. As the release of free arachidonic acid is the rate-limiting step in the biosynthesis of PGs (Flower and Blackwell, 1976), excess arachidonic acid (2 μg/ml) was added to each homogenate before incubation. Thus it can be assumed that any changes in PG production observed were not due to differences in arachidonic acid availability.

The following experiment was performed to determine that PG levels in rat aorta are low. PG metabolism in aortic homogenates is described in Section 4.2.

3.2.2 Methods

Four male rats were killed between 10.00 h and 12.00 h. The aorta of each rat was removed, weighed and homogenised in 5 ml absolute ethanol. The homogeniser was washed with 5 ml ethanol and the homogenate and washings were added to a centrifuge tube. Each homogenate was centrifuged at 1000 x g for 15 min and the supernatant fluid was retained. The precipitate was washed with 5 ml absolute ethanol, centrifuged at 1000 x g for 15 min and the resulting supernatant fluid was combined with the previous extract.

Each extract was evaporated to dryness, under reduced pressure at 45°C, then dissolved in 10 ml distilled water, PGs were extracted with ethyl acetate as described in Section 2.4, and were then stored and assayed by RIA as described in Section 2.5.

3.2.3 Results

The mean (+/- s.e.m., n=4) amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 in the aortic tissue of male rats are shown in Table 3.2.1, and have been compared with the amounts produced by homogenates during incubation.

Table 3.2.1

Concentration (mean+/- s.e.m.) of prostaglandins (PGs) in ethanolic homogenates (PG levels) and in homogenates of aorta from male rats, incubated with arachidonic acid (PG production).

	PG levels (n = 4)	PG production (n = 6)
6-keto-$PGF_{1\alpha}$	0.83 +/- 0.08 *	4.4 +/- 0.95
$PGF_{2\alpha}$	0.25 +/- 0.11 *	1.41 +/- 0.07
PGE_2	0.53 +/- 0.17	0.96 +/- 0.19

* Significantly different ($P<0.05$) from the amounts of PGs produced by homogenates incubated with arachidonic acid.

3.2.4 Conclusions

Concentrations of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 in aortae were low when compared to the amounts of PG formed during incubation of the homogenates with exogenous arachidonic acid, and this was significant for 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ but not for PGE_2. It can be concluded therefore, that the increased PG production found following homogenisation and incubation reflects fresh synthesis of PGs by the aortic tissue. It is probable that the other blood vessels behave similarly in this respect.

SECTION 3.3

Measurement of PG production in homogenates of aorta, vena cava, mesenteric artery and femoral artery of female rats.

3.3.1 Introduction

The sex steroids may be one of the controlling factors in arachidonate metabolism in vascular tissues. Uzonova et al., (1977) showed that arachidonic acid-induced thrombosis in mice is more prominent in the male than in the female and that castration of the male abolishes this effect. This occlusive arterial thrombosis was markedly reduced by cyclooxygenase inhibitors suggesting a central role for PGs. Similarly, experimentally induced arterial thrombosis in male as well as female rats was strikingly enhanced by testosterone treatment. Oestradiol treatment significantly increased the obstruction time, and decreased the thrombus weight in male rats but had no significant effect in the females (Uzonova et al., 1976). There is no biochemical mechanism given to explain these results but, if vascular PGI_2 were involved in these effects of steroids on thrombosis, it could be postulated that the male and female sex steroids were having an opposite effect on PGI_2 synthesis, with oestrogen stimulating and testosterone inhibiting its production. In contrast, however, Maggi et al. (1980) and Pomerantz et al., (1980) found a higher spontaneous release of PGI_2 from aortic rings of male as compared to female rats and castration had no effect on this release in male rats.

The experiments described in this section were undertaken to characterise the overall profile of PG production by different vascular tissues of the female rat. These results were then compared with the results obtained from the same vascular tissues of the male rats (detailed in Section 3.5).

3.3.2 Methods

Female albino Wistar rats, aged between 2 and 3 months and weighing 200-250 g were used. The animals were kept under controlled lighting (light period 06:00 h to 20:00 h) and were allowed free access to a standard diet and water. Vaginal smears were taken daily and examined microscopically. Day 1 (oestrus) was determined as the day of maximum cornification of the vaginal epithelial cells, preceeding the day of leucocyte infiltration. Only females showing at least 2 consecutive 4-day oestrous cycles were used in the study.

Animals (n = 6) were killed by stunning and incising the neck at 10:00 h on Day 4 (pro-oestrus). The females were always killed at this time and on Day 4 to eliminate any, as yet unknown, influences of the oestrous cycle on vascular PG production. The aorta and vena cava from each rat were dissected free from surrounding tissue and washed in Krebs solution. Another 6 females were similarly used to provide the mesenteric artery and the femoral artery. Each tissue was weighed and then homogenised separately in 5 ml Krebs solution. The

homogeniser was washed with 5 ml Krebs solution and the homogenate and washings were added to a 50 ml conical flask. The homogenates were incubated for 60 min with 2 μg/ml arachidonic acid. After incubation the pH of the homogenates was taken to pH 4 with 1N HCL, and the PGs were extracted with ethyl acetate as described in Section 2.4. Samples were stored in 4 ml ethyl acetate at -20°C until they were assayed for their 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 content by RIA as described in Section 2.5.

Results obtained from the male tissues were compared with those obtained from the female tissues using Student's t-test for unpaired data. Significance was tested at the 5% level.

3.3.3 Results (GC-MS)

The cross-reactivity studies given in Section 2.5 showed that the $PGF_{2\alpha}$ antiserum cross-reacted significantly with $PGF_{1\alpha}$ and that the PGE_2 antiserum cross-reacted significantly with PGA_2, PGB_2 and PGE_1. A pooled sample of aortic homogenates was therefore analysed further by GC-MS to identify positively the presence of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 and to investigate if there was sufficient PGA_2, PGB_2, PGE_1 or $PGF_{1\alpha}$ present to interfere with the RIA. Unfortunately there was insufficient sample from the other tissues for analysis by GC-MS. The procedure for the GC-MS analysis is given in Section 2.6.

Similar to the results obtained from male aorta, 6-keto-$PGF_{1\alpha}$ was positively identified in the pooled sample from female aorta and there may have been some PGE_2 present. Again, it was not possible to detect the presence of $PGF_{2\alpha}$.

Fig. 3.3.1 shows the amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 formed from exogenous arachidonic acid in homogenates of aorta, vena cava, mesenteric artery and femoral artery from Day 4 female rats. The aorta produced similar amounts of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ with less PGE_2, the vena cava produced mainly $PGF_{2\alpha}$ and PGE_2 with less 6-keto-$PGF_{1\alpha}$. The major PG produced by the mesenteric artery was 6-keto-$PGF_{1\alpha}$ followed, in descending order, by $PGF_{2\alpha}$ and PGE_2. The femoral artery produced mainly 6-keto-$PGF_{1\alpha}$ with less $PGF_{2\alpha}$ and PGE_2.

3.3.4 Conclusions

These experiments show that vascular tissues from female rats, like the vascular tissues from male rats, produce $PGF_{2\alpha}$ and PGE_2 as well as PGI_2 (measured as 6-keto-$PGF_{1\alpha}$). Production (per mg of tissue) of 6-keto-$PGF_{1\alpha}$ by the aorta and mesenteric artery was much greater than by the vena cava (3-fold) or femoral artery (2.5 fold). Thus, in the female rat, 6-keto-$PGF_{1\alpha}$ is the major PG produced by the mesenteric and femoral arteries with lesser quantities of $PGF_{2\alpha}$ and PGE_2, whereas in the aorta and the vena cava, $PGF_{2\alpha}$ production was greater than PGE_2 production and as great as 6-keto-$PGF_{1\alpha}$ production.

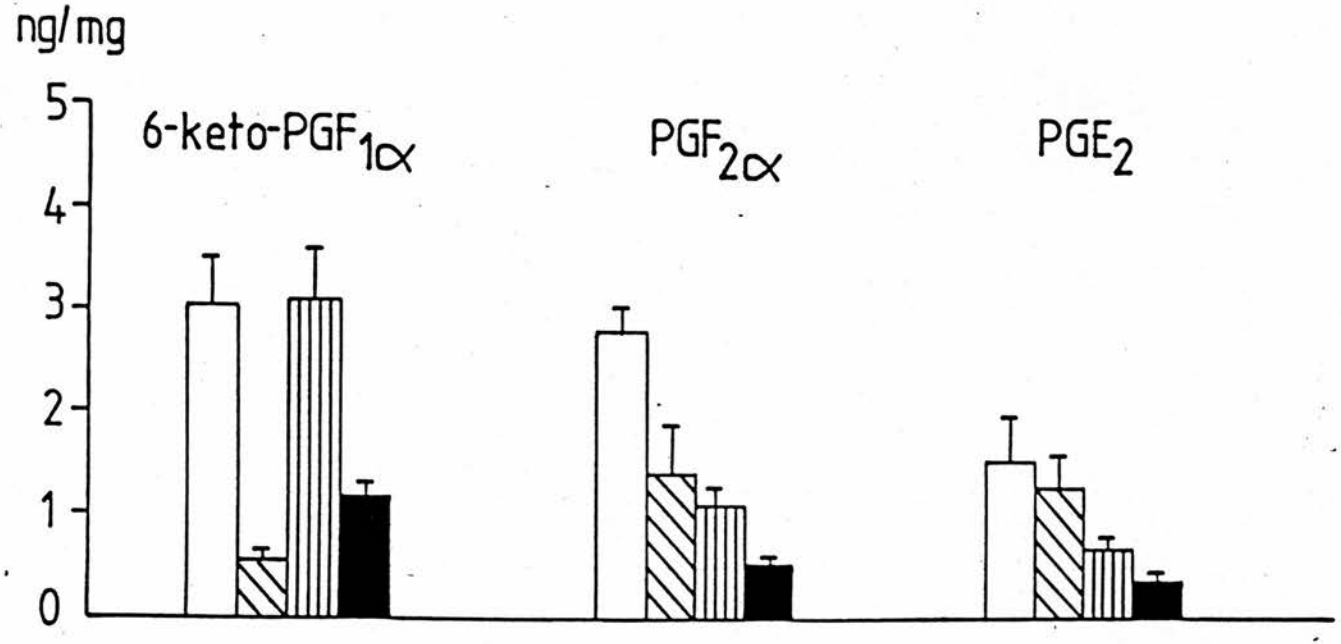


Fig. 3.3.1 Mean (+/- sem, n=6) amounts of prostaglandins (PGs) synthesised by homogenates of aorta, ☐ vena cava, ▨ mesenteric artery, ▥ and femoral artery, ■ of female rats.

SECTION 3.4

Measurement of aortic PG levels in female rats

3.4.1 Introduction

As described in Section 3.2, before the PG content of extracts of aortic homogenates incubated for 60 min can be termed PG 'synthesis' it is necessary to determine that before tissue homogenisation and incubation the levels of PGs are low, and that metabolism of the PGs is also low. The following experiment was performed to establish PG levels in the aorta of female rats.

3.4.2 Methods

Four female rats were killed on Day 4 of the cycle between 10:00 and 12:00 h. The aorta of each rat was removed, weighed and homogenised in 5 ml absolute ethanol. The homogeniser was washed with 5 ml absolute ethanol, and the homogenate and washings were added to a centrifuge tube. Each homogenate was centrifuged at 1000 x g for 15 min and the supernatant fluid was retained. The precipitate was washed with 5 ml absolute ethanol, centrifuged at 1000 x g for 15 min and the resulting supernatant fluid was combined with the previous extract.

Each extract was evaporated to dryness, under reduced pressure at 45°C, and dissolved in 10 ml distilled water.

PGs were extracted with ethyl acetate as described in Section 2.4, and were then stored at -20°C and assayed by RIA as described in Section 2.5.

3.4.3 Results

The mean (+/- s.e.m., n=4) amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 in the aortic tissue of female rats are shown in Table 3.4.1 and have been compared with the amounts produced by homogenates during incubation.

Table 3.4.1

Concentration (mean +/- s.e.m.) of prostaglandins (PGs) in ethanolic homogenates (PG levels) and in homogenates of aorta, from female rats incubated with arachidonic acid (PG production).

	PG levels (n=4)	PG production (n=6)
6-keto-$PGF_{1\alpha}$	0.71 +/- 0. 14 *	3.02 +/- 0.46
$PGF_{2\alpha}$	<0.04	2.82 +/- 0.20
PGE_2	0.17 +/- 0.002 *	1.52 +/- 0.44

* Significantly different ($P<0.05$) from the amounts of PGs produced by homogenates of aorta incubated with arachidonic acid.

3.4.4 Conclusions

Concentrations of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 in aortae were low when compared to the amounts of PG formed during incubation of the homogenates with exogenous arachidonic acid, and this was significant for 6-keto-$PGF_{1\alpha}$ and PGE_2. The concentrations of $PGF_{2\alpha}$ were so low as to be below the detection limit of the assay (0.04ng), therefore the amounts of $PGF_{2\alpha}$ produced by homogenates of aorta incubated with arachidonic acid were much greater. Thus, the increased PG production found following homogenisation and incubation reflects fresh synthesis of PGs by the aortic tissue.

3.4.5 Comparison of PG levels in and PG production by vascular tissues from male and female rats.

PG levels in aortic tissue were measured to establish that before homogenisation and incubation tissue levels of PGs were low. Table 3.4.2 shows the PG levels found in the aorta of male and female rats.

Table 3.4.2

Mean (+/- s.e.m., n = 4) concentration of prostaglandins (PGs) in ethanolic homogenates (PG level) of aorta from male and female rats.

	PG level (ng/mg tissue)	
	Male	Female
6-keto-$PGF_{1\alpha}$	0.83 +/- 0.08	0.71 +/- 0.14
$PGF_{2\alpha}$	0.25 +/- 0.11	<0.04
PGE_2	0.53 +/- 0.17	0.17 +/- 0.002 *

* Significantly different ($P<0.05$) from corresponding male value.

The total level of PGs present in aortic tissue before incubation were considerably lower in the female aorta compared to that of the male. This difference was not significant for 6-keto-$PGF_{1\alpha}$ but was significant ($P<0.05$) for PGE_2. $PGF_{2\alpha}$ levels in the female aorta were so low as to be below the detection limit of the assay. $PGF_{2\alpha}$ levels in male aorta were easily measurable and therefore much higher than in the female aorta.

PG production by blood vessels from male and Day 4 female rats is compared in Fig. 3.4.1. 6-keto-$PGF_{1\alpha}$ and PGE_2

production did not differ in the homogenates of aorta from male and female rats, but $PGF_{2\alpha}$ production was significantly ($P<0.05$) greater in the aorta of female rats. 6-keto-$PGF_{1\alpha}$ production was significantly greater ($P<0.05$) by the vena cava and was significantly lower ($P<0.05$) by the female artery of male rats compared to female rats. PGE_2 and $PGF_{2\alpha}$ production by the vena cava and by the female artery did not differ between male and female rats. The production of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 by the mesenteric artery did not differ between the 2 groups of male and female rats.

SECTION 3.5

Measurement of PG production by aorta and vena cava at different times during the oestrous cycle.

3.5.1 Introduction

The experiments to date on the effects of sex steroids on PG production have led to conflicting results. Stimulation of PGI_2 production by oestrogen has been described in cultured aortic smooth muscle cells (Chang et al., 1980). Oestrogen and a combined oestrogen/progestagen treatment also stimulated PGI_2 release from aortic rings (Karpati et al., 1980) and from tissue segments of aorta, vena cava, heart and lung (Roncaglioni et al., 1979). Conversely, Pomerantz et al., (1980) found that oestrogen suppressed and progesterone enhanced PGI_2 release from aortic rings.

Experiments described in this section were performed to determine if endogenous plasma levels of oestrogen and progesterone have an effect on PG production by aorta and vena cava. Female rats were killed at two different times during the oestrous cycle, 02:00 h on Day 1 (oestrus) and 10:00 h on Day 4 (pro-oestrus). Plasma oestrogen levels (oestradiol-17B) reach a peak on the morning of pro-oestrus (Day 4), and at 02:00 h on oestrus (Day 1) are approaching their lowest level. On the morning of pro-oestrus plasma progesterone levels are low but are just beginning to rise until they reach a peak somewhere around 22:00 h on that evening (Nequin, Alvarez and Schwartz, 1979). Thus, on the morning of pro-oestrus, the effects of an endogenously high plasma oestrogen and low plasma progesterone level could be studied, and on the early morning of oestrus, the converse of a low plasma oestrogen and high plasma progesterone level could be investigated with regard to vascular PG production.

3.5.2 Methods

Female albino Wistar rats, aged between 2 and 3 months and weighing 200-250 g were used. The animals were kept under controlled lighting conditions (light period 06:00 h to 20:00 h) and allowed free access to a standard diet and water.

Animals were killed by stunning and incising the neck at 10:00 h on Day 4 (pro-oestrus) and 02:00 on Day 1 (oestrus) 6 animals were used for each day. The aorta

and vena cava from each rat were removed, and homogenised and incubated as detailed in Section 3.1.1. The PGs produced were extracted with ethyl acetate and stored until assayed by RIA, as described in detail in Section 2.5. Results were analysed using Student's t-test for unpaired data and significance was tested at the 5% level.

3.5.3 Results

Figure 3.5.1 shows the amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 formed from exogenous arachidonic acid (2 μg/ml) in homogenates from aorta and vena cava at 10:00 h on Day 4 and at 02:00 on Day 1. 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were produced in greatest amounts by aortic homogenates from both Day 1 and Day 4 rats, with lesser amounts of PGE_2 being produced. Homogenates of vena cava produced mainly $PGF_{2\alpha}$ and PGE_2, with lesser amounts of 6-keto-$PGF_{1\alpha}$. There were no significant differences in the production of any one PG by homogenates of aorta and vena cava between rats on Day 1 and Day 4 of the oestrous cycle.

3.5.4 Conclusions

At the two periods of the oestrous cycle studied here, of high and low endogenous plasma oestrogen levels, and low and high endogenous plasma progesterone levels, there was no significant effect on PG production in either the aorta or vena cava, suggesting that the stimulatory effects of oestrogen observed in tissue culture

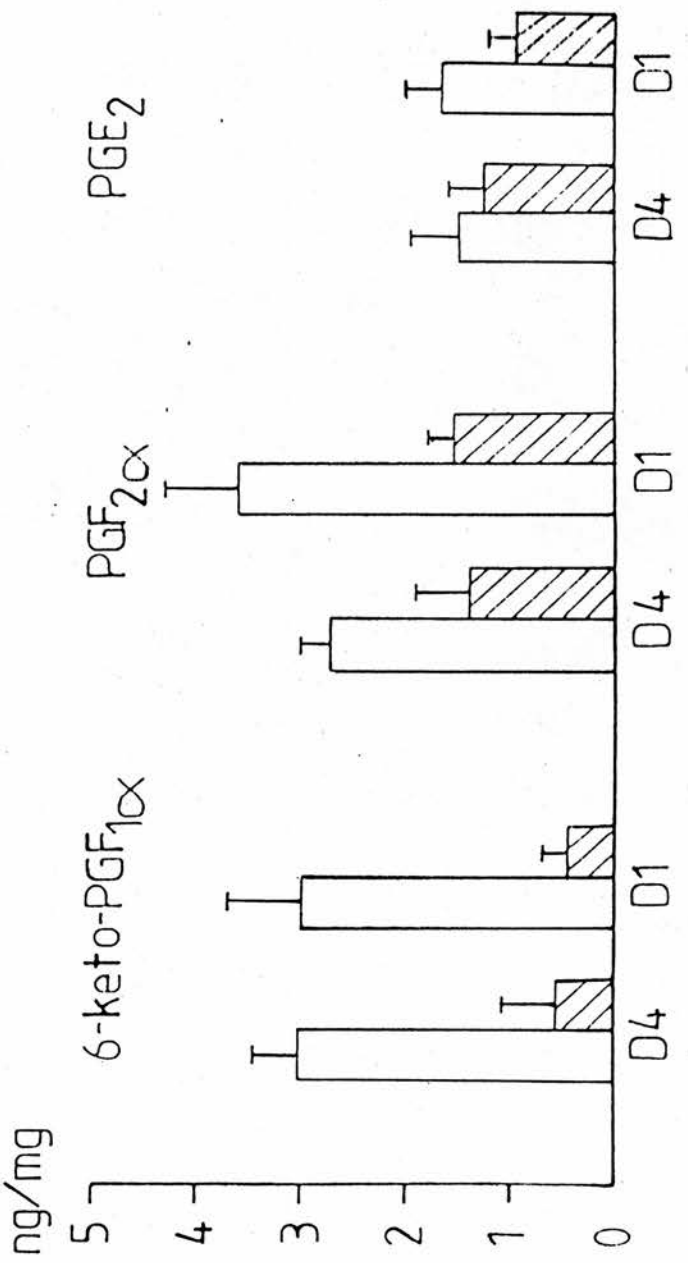


Fig. 3.5.1. Mean (+/- sem, n=6) amounts of prostaglandins (PGs) synthesised by homogenates of aorta ☐ and vena cava ▨ from female rats on the days of pro-oestrus (D4; 10:00 h) and oestrus (D1; 0.2:00 h).

experiments or after long-term treatment in the intact animal are pharmacological in nature. To test this hypothesis, the effects of oestradiol and progesterone on PG production by the blood vessels of ovariectomised rats was investigated next.

SECTION 3.6

Measurement of PG production by aorta and vena cava of oestrogen and progesterone treated females.

3.6.1 Introduction

Studies by other research workers have investigated the effects of long-term treatment with oestrogen or an oestrogen/progestagen combination on vascular PG production. Karpati et al. (1980) and Roncaglioni et al. (1979) designed their experiments specifically to investigate the effects of long-term treatment with a synthetic oestrogen/progestagen combination. These studies were all performed in intact rats, and since no atttempt has been made to mimic or consider the animals' natural production of steroids by the ovary, the following experiment was undertaken using a more physiological model of oestrogen and progesterone treatment, which was designed to simulate the plasma steroid hormone concentrations occurring around ovulation. (Karla, Fawcett, Krulich and McCann, 1973).

3.6.2 Methods

Female albino Wistar rats weighing between 200 and 250 g were kept under controlled lighting conditions and allowed free access to food and water. Twelve rats were ovariectomised between 09:00 h and 10:00 h on di-oestrus (Day 3), under Althesin anaesthesia (0.5 ml / 100 g body weight, i.p). Six of these animals received 10 μg oestradiol benzoate in 0.5 ml of arachis oil as a subcutaneous injection (s.c.). The other six animals were injected with 0.5 ml of the arachis oil vehicle. On the following day, the expected pro-oestrus, the animals were injected at 10:00 h with 2 mg progesterone s.c. in 0.5 ml arachis oil. At 17:00 h, five hours after the progesterone injection, the rats were killed and their aorta and vena cava dissected out, weighed, homogenised and incubated as described previously in Section 2.4. The PGs produced were extracted and assayed by RIA as described in Section 2.5. Results were compared by means of Student's t-test for unpaired data and significance was tested at the 5% level.

3.6.3 Results

Fig. 3.6.1 compares PG production by homogenates of aorta and vena cava from acute ovariectomised, oestrogen and progesterone treated rats and control, ovariectomised rats. 6-keto-$PGF_{1\alpha}$ was the major PG produced by the aorta of both treated and control animals, followed in descending order by $PGF_{2\alpha}$ and PGE_2. In both groups $PGF_{2\alpha}$ and PGE_2a were the major PGs produced by homogenates of vena cava with lesser amounts of 6-keto-$PGF_{1\alpha}$. There

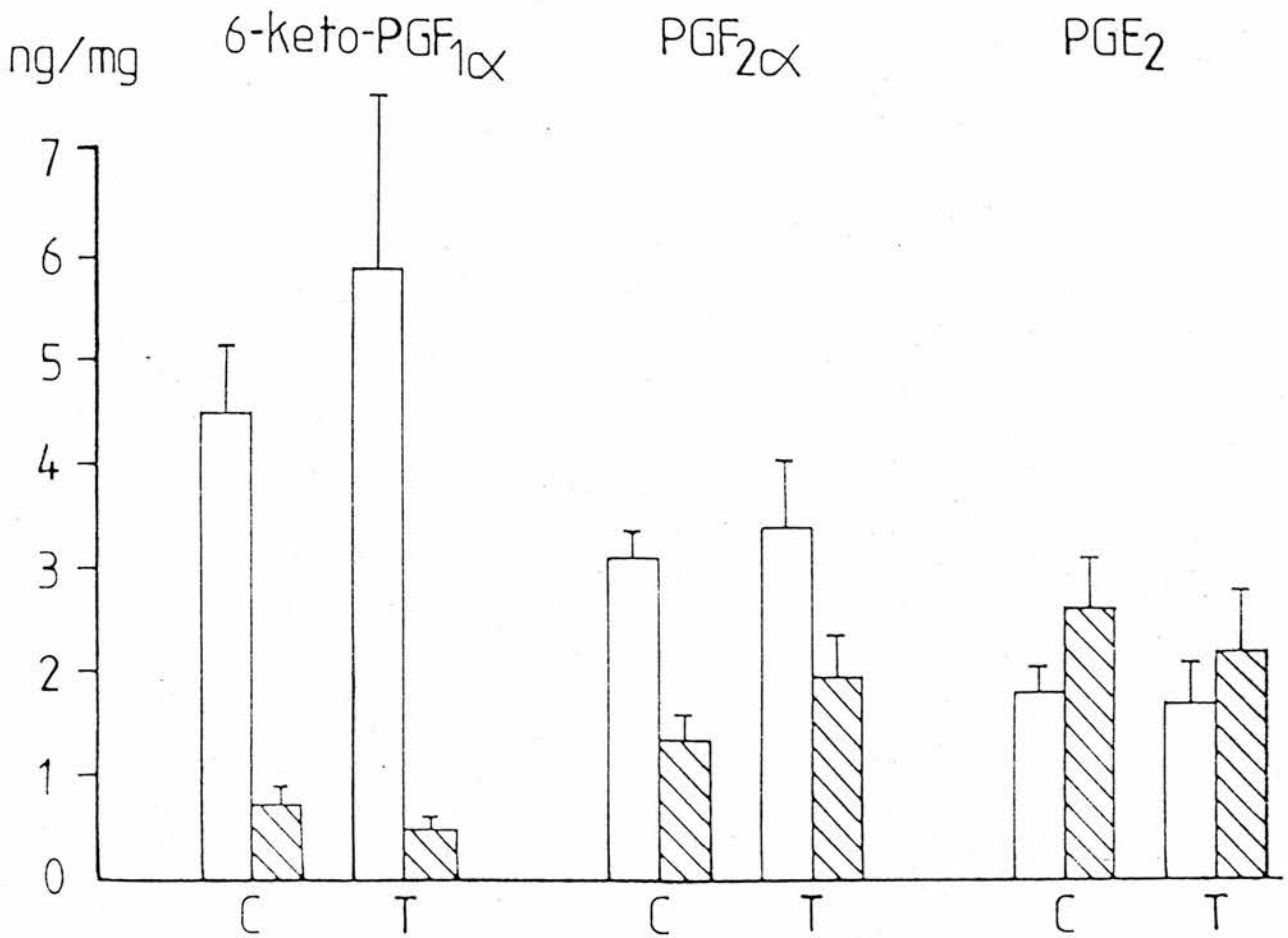


Fig. 3.6.1. Mean (+/- sem, n=6) amounts of prostaglandins (PGs) synthesised by homogenates of aorta □ and vena cava ▨ from acutely ovariectomised rats without (C) and with (T) oestradiol and progesterone treatment.

were no significant differences in the production of any one PG by either the aorta or vena cava of oestrogen and progesterone-treated rats compared to control, ovariectomised rats.

However, the procedure of ovariectomy in itself appears to have had a significant effect on vascular PG production. Comparing intact Day 1 or Day 4 rats to ovariectomised control or treated rats there was a significant ($P<0.05$) increase in 6-keto-$PGF_{1\alpha}$ production by aorta from ovariectomised rats, and a significant increase in PGE_2 production by vena cava from ovariectomised compared to intact rats.

3.6.4 Conclusions

A model of oestrogen and progesterone treatment, involving short-term ovariectomy and 2 days of steroid treatment, designed to simulate the steroid changes found at ovulation did not influence PG production by the aorta or vena cava. These results agree with the findings of the previous sub-section that changes in plasma levels of oestrogen and progesterone in the female rats do not stimulate PG production by these vascular tissues. One observation of interest is that the profile of PG production in rats ovariectomised for 33 h is different to that of the intact animal. Deprivation of steroid hormones may therefore have affected PG production by the blood vessels, suggesting that these hormones do have a long-term, as opposed to a short-term effect on vascular PG production. Short-term treatment with steroids did not reverse the effects of ovariectomy.

3.7 Discussion

The experiments described in this section have established that several vascular tissues from male and female rats have the capacity to produce $PGF_{2\alpha}$ and PGE_2 as well as PGI_2 (measured as 6-keto-$PGF_{1\alpha}$). With the exception of the aorta in the male rat and the mesenteric artery in the female rat the synthetic capacity of the blood vessels for producing these two vasoactive PGs was as great as for PGI_2. Recent studies have verified that vascular cells have the ability to release PGs other than PGI_2, with 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 shown to be produced by porcine endothelial cells in culture (Ody et al., 1983) and by rabbit blood vessels (Foerstermann et al., 1984; Boeynams and Galand, 1983).

In rabbit, rat and dog, arteries have been shown to produce, and also release more PGI_2 than veins (Buchanan et al., 1979; Skidgel and Printz, 1978; Eldor et al., 1981) but no difference in PG production by bovine arteries and veins was detected (Terragno et al., 1975). In the present study, aortic 6-keto-$PGF_{1\alpha}$ production was certainly greater than that of the vena cava. However, in the male rat, 6-keto-$PGF_{1\alpha}$ production by the mesenteric and femoral arteries was not significantly greater than by the vena cava, although it was greater in the female.

In dogs, a segment of jugular vein implanted into the carotid artery for up to 6 weeks, although becoming 'arterialised' from a structural point of view, maintained a lower production of PGI_2 than the carotid artery (Eldor et al., 1981). However, a similar study in rats, implanting a segment of epigastric vein into the femoral artery showed a progressive rise of PGI_2 production by the graft from venous levels to values close to the arterial controls (Petry et al, 1982). This suggests that in the rat at least, PGI_2 production may be related to blood pressure and flow.

Contrary to the findings of Pomerantz et al. (1980) and Wey et al. (1983), the aorta from male rats did not produce more 6-keto-$PGF_{1\alpha}$ than the aorta from females, but their studies were looking at 6-keto-$PGF_{1\alpha}$ release from aortic rings rather than synthetic capacity as measured here. However, 6-keto-$PGF_{1\alpha}$ production was greater by male vena cava than by female vena cava.

No difference in PG production was observed at 10:00 *h* on pro-oestrus and 02:00 h on oestrus. Ali and Williams (1983), found that PGI_2 production was lowest at oestrus and significantly greater at pro-oestrus, met-oestrus and di-oestrus, showing a similar fluctuation to the PGI_2 production of the myometrium. These results were obtained in the presence and in the absence of arachidonic acid but the authors do not record at what time of the day their experiments were carried out. The inability of the present study to detect any change in PG

production during the cycle may have been because the times chosen did not coincide with any increase in PG production. Further experiments are required to clarify this point.

There are conflicting results regarding the influence of sex steroids on PGI_2 production. In the present investigation short-term treatment with oestrogen and progesterone after acute ovariectomy had no effect on the production of 6-keto-$PGF_{1\alpha}$ by aorta or vena cava. This agrees with the observation that PG production does not vary during the periods of high and low plasma oestrogen levels during the oestrous cycle. However, oestrogen treatment significantly inhibits the thrombus formation induced by electric shock with the concomitant stimulation of PGI_2 synthesis in the aorta (Ohtsu et al., 1983). Thus, providing a biochemical mechanism for the findings of Uzonova et al. (1976) who found that oestrogen treatment increased the obstruction time and reduced the thrombus weight of male mice injected with arachidonic acid.

Oestrogen also stimulated PGI_2 production in cultured rat aortic smooth muscle cells (Chang et al., 1980) and from cultured porcine endothelial cells from female animals (Seillan et al., 1983). Stilboestrol treatment of ovariectomised rats stimulated aortic PGI_2 production (Ali and Williams, 1983) and neonatal oestrogen administration to female rats resulted in an increased

capacity for 6-keto-$PGF_{1\alpha}$ synthesis in adult aortae (Wey et al., 1983). Conversely, oestrogen inhibited (Needleman and Parks, 1982) or had no effect (Corvazier, Dupuy, Dosne and Maclouf, 1984) on PGI_2 production by human umbilical vein endothelial cells in culture.

In the present study acute ovariectomy produced a significant increase in 6-keto-$PGF_{1\alpha}$ production by the aorta in agreement with Wey et al., (1983) who found that neonatal ovariectomy increased the capacity of the aorta for 6-keto-$PGF_{1\alpha}$ synthesis, and Pomerantz et al., (1980) who found that ovariectomy at 3 weeks of age increased the aortic synthesis of 6-keto-$PGF_{1\alpha}$ in the adult rat. Therefore, in this situation, the absence of oestrogen appears to stimulate PGI production. Testosterone has been reported to inhibit PGI_2 production by rat aortic smooth muscle cells (Nakao et al., 1981) or to have no effect (Pomerantz et al., 1980; Wey et al., 1983; Seillan et al., 1983).

The relationship of sex hormones and PGI_2 production and their relation to thrombosis is complex and the work of Uzonova et al. (1976) showing that male rats have twice the thrombus size and death rate compared with females and that oestrogen reduced the thrombus weight in male rats can not be explained simply by the conflicting results of the effects of steroid hormones on PGI_2 production. Other factors to be taken into consideration appear to be the hormonal background of the experimental model chosen, whether the animal has or has not been

ovariectomised, or, in the case of the umbilical vein endothelial cells, whether the hormonal milieu of pregnancy has affected the response of the tissue to steroid hormones.

SECTION 4.0

Measurement of the PG synthetic capacity of homogenates of aorta, the basal release of PGs from the perfused aorta and the stimulated release of PGs from the perfused mesenteric arterial bed of male and female normotensive and New Zealand genetically hypertensive rats.

Introduction

The level of arterial blood pressure is set by complex interactions of several mechanisms which influence blood flow and resistance of the vascular system. In hypertension the final common pathway that leads to a raised arterial pressure is an increased total peripheral resistance. This is regardless of the underlying cause such as increased activity of the sympathetic nervous system, of the renin-angiotensin system, or excessive secretion of mineralocorticoids. One of the earliest known properties of PGs was their effect on blood pressure, and many of the current studies on hypertension are aimed at determining the association of PGs with blood pressure regulation. The hypothesis that PGs could be involved in these mechanisms has gained strength with the demonstration that the kidney and blood vessels are major sites of PG synthesis, that pressor hormones induce the release of PGs and that PGs are probably involved in the renal mechanisms affecting blood pressure control.

The hypotensive action of PGs of the E series has been known for a long time, and it is now well established that PGE_2 inhibits the release of NA from many sympathetic nerves. This latter action has been shown in various species and in several different preparations (for review see Hedquist, 1977). The effect of PGI_2 on transmitter release from sympathetic nerves is contradictory. Hedquist (1979) found that PGI_2 had little or no effect on [^{3}H]-noradrenaline release in response to nerve stimulation in rabbit kidneys, but Weitzell et al. (1978) reported inhibition of NA release by PGI_2 in rabbit pulmonary arteries. Armstrong et al. (1979) observed a similar effect with PGI_2 in rabbit mesenteric arteries, but not veins.

It has been proposed that a deficiency of production of vasodilator PGs in vascular beds, (particularly the kidney) may lead to the increased peripheral vascular resistance characteristic of essential hypertension (Vane and McGiff, 1975). However, several groups have reported that the generation of PGI_2 and PGE_2-like substances from exogenous arachidonic acid is much greater in aortic rings and homogenates from spontaneously hypertensive rats (SHR) than in aorta from normotensive rats (Limas and Limas, 1977; Rioux et al., 1977; Pace-Asciak et al., 1978). The release of a PGI_2-like substance was also greater from aortic rings of stroke-prone and stroke-resistant SHR compared with normotensive controls (Okuma et al., 1980) and was greater from aortic strips of the New Zealand genetically hypertensive (GH) strain compared to normotensive rats (Botha et al., 1980).

Because of the equivocal nature of the results obtained to date on the role of vascular PGs in the control of blood pressure, it seemed necessary to investigate PG production by blood vessels in the hypertensive rat in greater detail. PGI_2 is the PG most often measured, while PGE_2 and $PGF_{2\alpha}$ are often completely overlooked.

The results described in the previous section indicate a considerable capacity of rat blood vessels for producing PGE_2 and $PGF_{2\alpha}$, therefore, experiments described in this section aimed to characterise more complete profiles of PGI_2, PGE_2 and $PGF_{2\alpha}$ production in separated layers of aorta, of the release of these 3 PGs from perfused aorta, and of the stimulated release of these 3 PGs from the mesenteric arterial bed of male and female normotensive and hypertensive (GH) rats.

SECTION 4.1

Measurement of the PG synthetic capacity of homogenates of aorta and vena cava from normotensive and genetically hypertensive male rats.

4.1.1 Introduction

The experiments described in this section were undertaken to establish the blood pressure of the control normotensive Wistar rats and of the New Zealand genetically hypertensive rats (GH) which had been kept as a colony for a number of years in the departmental animal house. The ability of homogenates of aorta and vena cava from GH rats to synthesise PGs was compared with that of normotensive Wistar control male rats.

4.1.1 Methods

Male albino Wistar rats aged 2-3 months and weighing 200-250 g and male New Zealand genetically hypertensive rats (GH) of the same age, weighing 180-200 g were used. The animals were housed under controlled conditions and were allowed a standard diet and water.

4.1.3 Blood pressure measurement.

Measurements were made in the same cage order and the same time of day (11:00 h to 13:00 h), every day for 2 weeks. Systolic blood pressure was measured indirectly

using the tail cuff method. Pulsations of the tail artery in pre-warmed rats were monitored by a photo-electric detector (Huntingdon Instruments Rat Blood Pressure Monitor) and recorded on a Servoscribe Two-channel Recorder, together with cuff pressure.

Rats were pre-warmed using heating lamps. The animals were then placed in a heated (37 °C) holder and lightly restrained. The tail cuff was applied firmly to the tail but without constricting the artery, and 5 consecutive readings of systolic pressure were obtained. Blood pressure was determined by rapidly increasing the pressure in the cuff until the tail artery pulse disappeared, then gradually releasing the pressure over 10-15 sec and noting the pressure at which the pulsations first reappeared. This value was taken as the systolic blood pressure. After about a week, the rats were trained in this routine and consistent readings were obtained.

After consistent blood pressure measurements had been made, the animals were killed (6 in each group) and the aorta and vena cava were removed. Each vessel was weighed, was homogenised separately and was incubated for 60 min with arachidonic acid (2 μg /ml). The PGs produced were then extracted into ethyl acetate (as described in Section 2.4) and stored until assayed for 6-keto-$PGF_{1\alpha}$, PFG2a and PGE_2 content by RIA as detailed in Section 2.5.

4.1.4 Results

The systolic blood pressure of the normotensive rats was 104.8 +/- 4.3 mmHg (mean +/- s.e.m.; n = 6) and that of the GH rats was 150.5 +/- 3.1 mmHg (mean +/- s.e.m.; n=6).

Figure 4.1.1 compares PG production by homogenates of aorta and vena cava from male normotensive and GH rats. 6-Keto-$PGF_{1\alpha}$ was the major PG produced by aorta of normotensive and GH rats followed by lesser amounts of $PGF_{2\alpha}$ and PGE_2. The vena cava from normotensives produced similar amounts of the 3 PGs whereas the vena cava from GH rats tended to produce more $PGF_{2\alpha}$.

There was no difference in the amounts of either 6-keto-$PGF_{1\alpha}$ or PGE_2 produced between the 2 groups. However, the amount of $PGF_{2\alpha}$ produced was significantly greater ($P < 0.05$) by homogenates of aorta from GH rats compared to normotensive rats. $PGF_{2\alpha}$ production by the vena cava tended also to be higher between the two groups but the difference was not statistically significant ($P > 0.05$).

4.1.5 Conclusions

Although the systolic blood pressure of the GH rats was not as high as reported by others (Simpson, Phelan, Jones, Butt, Young and Ledingham, 1979) it was significantly higher ($P < 0.01$) than that of the

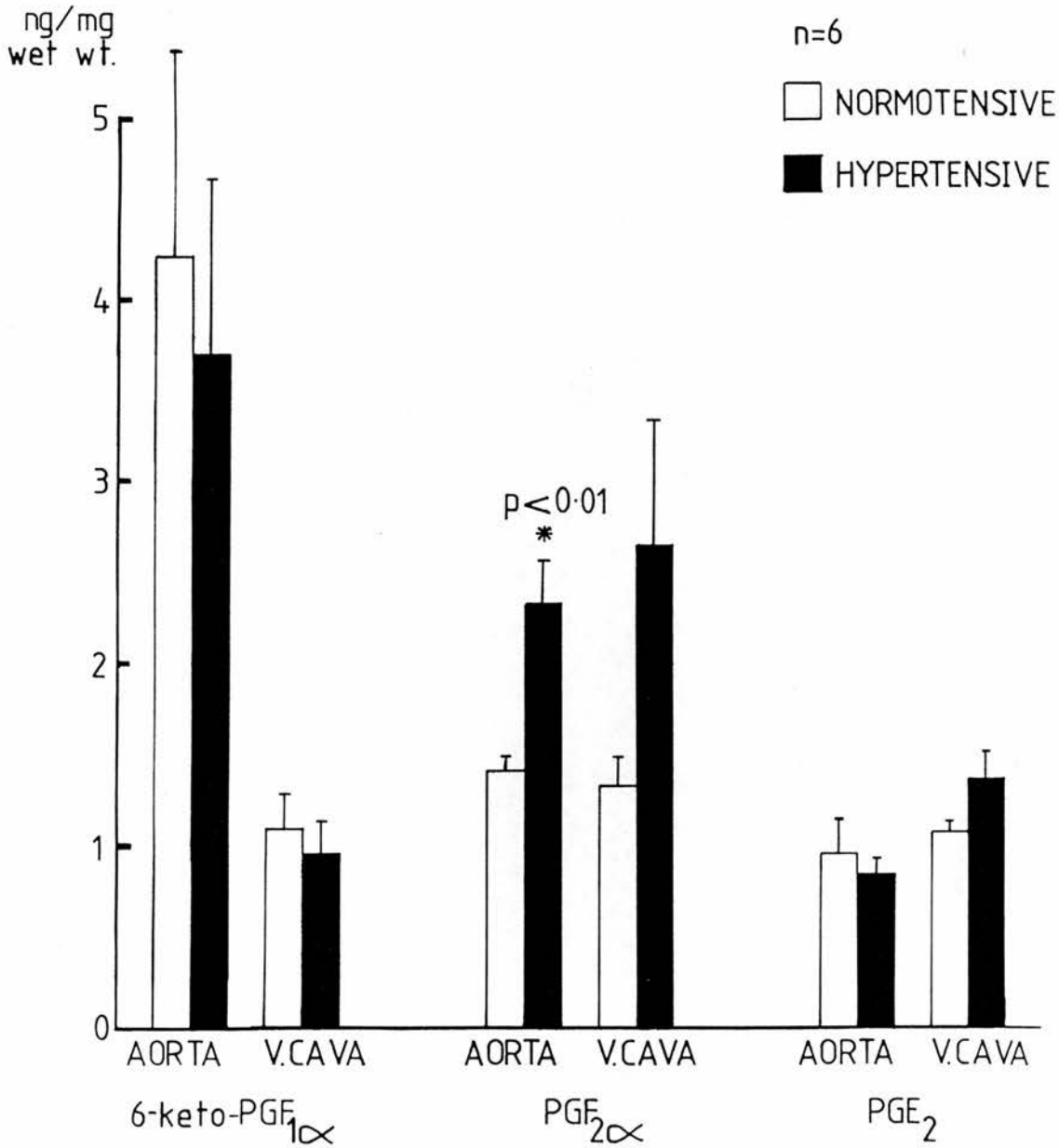


Figure 4.1.1. Mean (+/- s.e.m., n=6) amounts of prostaglandins (PGs) synthesised by homogenates of aorta and vena cava from normotensive and New Zealand genetically hypertensive male rats.

normotensive rats. The lower blood pressure found in these GH rats was probably as a result of the colony not being as stringently maintained as possible i.e. with continual brother to sister mating of the animals with the highest blood pressure. However, all of the GH animals measured did have a markedly higher blood pressure than that of their normotensive controls.

PGI_2 production, measured as 6-keto-$PGF_{1\alpha}$, was not significantly greater in either homogenates of aorta or vena cava of normotensive compared to GH rats which does not agree with the findings of Limas and Limas, (1977); Rioux et al. (1977); or Pace-Asciak et al., (1978) but their studies were carried out in the spontaneously hypertensive rat (SHR) not in the New Zealand GH rat as in this study. The most striking difference between the 2 groups was the increased production of $PGF_{2\alpha}$ by homogenates of aorta and vena cava of hypertensive compared to normotensive rats. This observation has not been reported previously.

SECTION 4.2

Measurement of the ability of aortic homogenates to metabolise $PGF_{2\alpha}$.

4.2.1 Introduction

Augmented production and release of PGs from a tissue may indicate either enhanced synthesis and/or diminished degradation of PGs, as there is no appreciable storage of

PGs in tissues. The enzyme 15-prostaglandin hydroxydehydrogenase (15-PGHD) is responsible for the catabolism of PGE_2 and $PGF_{2\alpha}$ and this enzyme has been shown to be present in both arteries and veins (Wong and McGiff, 1977). It can also inactivate PGI_2 by converting it to 6, 15 di-keto $PGF_{1\alpha}$ (Sun et al., 1979).

A deficiency of 15-PGHD activity has been found in the renal vessels of New Zealand GH rats (Armstrong et al., 1976) and also in SHR (Pace-Asciak, 1976). An endogenous inhibitor of 15-PGHD has been recovered from the kidney of GH rats (Wong, Baer, McGiff, 1979). If the kidney of GH rats shows an aberration in 15-PGHD activity it is possible that this defect is also present in the 15-PGHD from other blood vessels, so the metabolism of $[^3H]$ $PGF_{2\alpha}$ was investigated in homogenates of aorta from normotensive and GH male rats.

4.2.2 Methods

Four normotensive and four GH male rats (2-3 months old) were used. The animals were killed, the aortae were removed, and each aorta was cut in half and weighed. Each half aorta was then homogenised in 5 ml of Krebs solution. Nicotinamide-adenine dinucleotide (NAD^+) at a final concentration of 2 mM and 10 μg of $PGF_{2\alpha}$ were added to one of the aortic halves. Each homogenate was then incubated in the presence of 0.5 μCi of $[^3H]$ $PGF_{2\alpha}$ (sp.act.160 Ci/mmol) for 60 min. The PGs were extracted as described in Section 2.4. The extracts were taken to

dryness at 45°C under reduced pressure and the residues were redissolved in 0.2 ml of methanol and subjected to thin layer chromatography (TLC).

Non-radioactive marker standards (10 μg) of $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ were spotted onto a pre-coated silica gel TLC plate (Merck, U.K.). Sample extracts were also streaked onto separate TLC plates. The plates were developed in the solvent system, chloroform: methanol:glacial acetic acid: water, 90:9:1:0.65, to give a solvent front 15 cm from the origin. Standard spots on the control plates were developed in iodine vapour for 30 min and the Rf values were calculated. Experimental plates were scanned using a Panax radio TLC plate scanner to localize the radioactivity. The plates were marked at 0.5 cm intervals and the silica gel was scraped off the plates into scintillation vials. Methanol (1 ml) was added to each vial followed by 10 ml scintillation fluid (10.5 g PPO, 1.5 l toluene). Radioactivity was monitored in a Philips scintillation counter using sample channels ratio counting. The Rf values of radioactive peaks on the plate were compared to Rf values of standard $PGF_{2\alpha}$ and metabolites. Due to the close proximity of the two $PGF_{2\alpha}$ metabolites on the TLC plate, they were not separated for counting purposes and the percentage metabolism of $PGF_{2\alpha}$ into these two products was expressed as a single percentage of the total radioactivity on the plate.

4.2.3 Results

The Rf values for the non-radioactive marker standards are shown in Table 4.2.1.

Table 4.2.1

Rf values of non-radioactive marker standards

Compound	Rf value
$PGF_{2\alpha}$	0.18
15-keto-$PGF_{2\alpha}$	0.43
13,14-dihydro, 15-keto-$PGF_{2\alpha}$	0.53

Table 4.2.2 shows the percentage metabolism of $PGF_{2\alpha}$ in aortic homogenates of male normotensive and GH rats.

Table 4.2.2

Percentage metabolism of $PGF_{2\alpha}$ in aortic homogenates of normotensive and GH male rats, incubated in the absence and presence of NAD^+ (mean +/- s.e.m.).

	Normotensive		Hypertensive	
	Without NAD^+	With NAD^+	Without NAD^+	With NAD^+
Total metabolism	17.1+/-2.5	17.8+/-1.9	15.2+/-2.4	17.1+/-2.3
Metabolism per 10 mg tissue	3.5+/-0.7	4.0+/-0.7	3.5+/-0.6	5.0+/-1.2

Incubation of homogenates of aorta resulted in the formation of metabolites with Rf values corresponding to the 15-keto and 13, 14-dihydro-15-keto metabolites of $PGF_{2\alpha}$. The percentage metabolism of $PGF_{2\alpha}$ in the absence of NAD^+ was low and did not increase significantly with the addition of NAD^+, in either group. The percentage metabolism of $PGF_{2\alpha}$ did not differ significantly between homogenates of aorta from normotensive compared to GH rats.

4.2.4 Conclusions

Metabolism of $PGF_{2\alpha}$ in aortic homogenates in the absence and presence of NAD^+ is low. As blood vessel PG levels are also low, it can be concluded that the amounts of PG

produced by incubating these homogenates with arachidonic acid reflect the PG-synthesising capacity of the vascular tissue.

Metabolism of $PGF_{2\alpha}$ in homogenates of aorta from GH rats was not significantly lower than that in homogenates from normotensive, suggesting that the increased amounts of $PGF_{2\alpha}$ measured in aorta and vena cava from hypertensive animals are due to increased production of $PGF_{2\alpha}$ and not due to a decrease in its metabolism.

As the same enzyme, 15-PGHD is responsible for the catabolism of PGE_2 and PGI_2 it is probable that, in the absence of NAD^+, metabolism of these PGs by homogenates is also low. Another enzyme, 9-prostaglandin E ketoreductase (9-PGEKR) catalyses the stereospecific reduction of PGE_2 to $PGF_{2\alpha}$ (Leslie and Levine, 1973). The activity of 9-PGEKR was not investigated in this study but it is probable that the increased amounts of $PGF_{2\alpha}$ found in homogenates of aorta from GH rats is not due to increased conversion of PGE_2 to $PGF_{2\alpha}$, as PGE_2 production was not significantly reduced in aortic homogenates of GH compared to normotensive rats.

SECTION 4.3

Measurement of PG production in the separate layers of aorta from normotensive and GH male rats.

4.3.1 Introduction

The lack of reactivity between platelets and vascular endothelium has been known for many years. However, when sub-endothelial layers are exposed, platelet aggregation is strongly stimulated. The reasons for the absence of platelet aggregation in the presence of an intact endothelial layer were unknown until the discovery of an enzyme for the synthesis of the potent vasodilatory and anti-aggregatory PG, PGI_2, in rabbit blood vessel. (Moncada et al., 1976). These authors postulated that PGI_2 might be formed from endoperoxides released by the platelets, thus formulating an hypothesis of endothelial cell and platelet interaction (discussed in detail in Introduction section).

Subsequently, PGI_2-like activity, measured by platelet bioassay, was found to be produced by all layers of the rabbit thoracic aorta. The amount of PGI_2 formed was found to decrease progressively from the endothelial layer to the adventitia (Moncada et al., 1977).

The technique of cell culture has since been used to identify and quantitate the PGs produced by vascular endothelial and smooth muscle cells. However, changes in

the pattern of PG production during culture have been observed in pig aortic endothelial and smooth muscle cells (Ager et al., 1979 & 1982) and in endothelial cells from human artery (Siess et al., 1981). Differences have also been observed in the absolute amounts of PGE_2 and PGI_2 produced from fresh tissue compared to cultured cells (Ager et al., 1982; Goldsmith, et al., 1982).

In this set of experiments therefore, the previous technique of separation of the vessel layers, followed by homogenisation and incubation of the layers with AA has been employed to investigate the profile of PG production by the aorta of normotensive and GH male rats.

4.3.2 Methods

Male albino Wistar rats weighing 250g-300g (2-3 months old) and male GH rats of the same age, weighing 200-250 g were used.

Preparation of the different layers of the vessel wall

The method of Moncada et al. (1976), using rabbit aorta was followed with some modifications. Six animals from each group were killed and the aorta carefully dissected from surrounding tissue. The aorta was rinsed in Krebs solution to remove any blood, was opened longitudinally and, with its endothelial surface uppermost, was pinned out on a plastic petri-dish. The endothelial cells were carefully scraped off with a scalpel blade and were

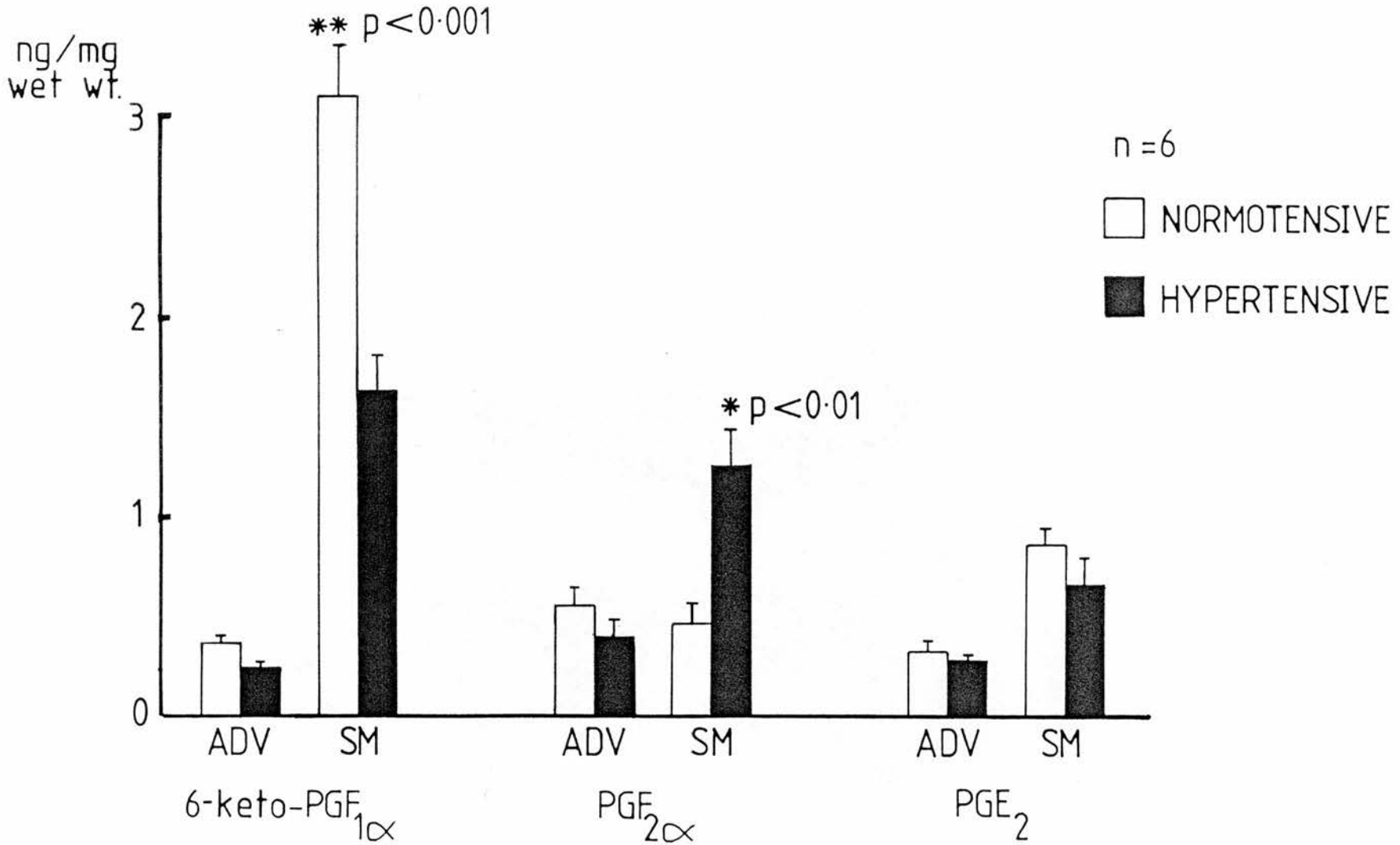
suspended in 5 ml Krebs solution. The adventitia and the smooth muscle layer were also separated with a scalpel blade and then weighed.

The adventitia and smooth muscle layers were homogenised separately in 2.5 ml Krebs solution. The homogeniser was washed with 2.5 ml Krebs solution and the homogenate and washings were added to a 25 ml conical flask. The endothelial cell layer was left as a cell suspension. An aliquot portion (100 μl) from each of the three layers was kept for determination of protein content according to the method of Lowry et al. (1951). After 2 μg/ml of arachidonic acid had been added to each flask, the flasks were incubated for 60 min and the PGs produced were extracted with ethyl acetate as described in Section 2.4. Samples in ethyl acetate were stored at -20°C until assayed for PG content by RIA as detailed in Section 2.5.

4.3.3 Results

Figure 4.3.1 details PG production by homogenates of adventitia and smooth muscle from the aorta of normotensive and GH male rats. The results are expressed as ng of PG per mg of tissue rather than ng of PG per mg of protein because the smooth muscle layer from the aorta of GH rats was found to contain a significantly higher ($P < 0.001$) amount of protein per mg of wet weight than that of normotensives, this difference distorted the results when expressed per mg of protein. Also, there was insufficient protein present in the 100 μl aliquot

Figure 4.3.1. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by homogenates of adventitia (ADV) and smooth muscle (SM) from normotensive and New Zealand genetically hypertensive male rats.

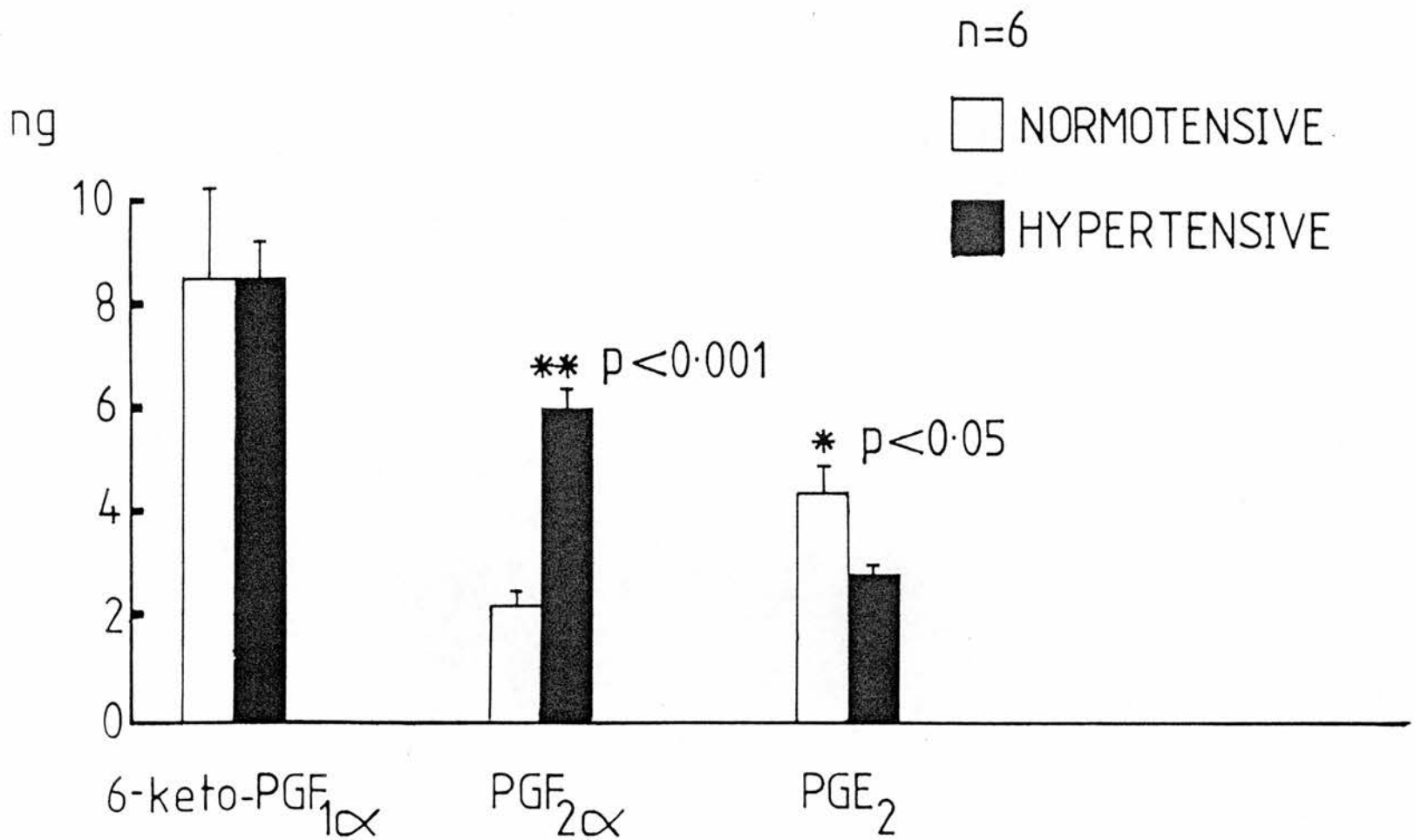


portion from the 5ml endothelial cell suspension to determine accurately enough the protein concentration. Thus, a direct comparison of the PG synthetic capacity (per mg protein or per unit weight) of the three different layers was not possible. However, a comparison of the synthetic capacity of the different layers for PG production, expressed as total PG production by a 2.5 cm length of aorta is shown in Table 4.3.1.

The results per mg wet weight of tissue will be discussed first. Adventitia from normotensive and GH rats produced similar amounts of the 3 PGs and there was no significant difference in PG production between the two groups. The smooth muscle layer had a much greater capacity for PG production than the adventitia. 6-keto-$PGF_{1\alpha}$ was the major PG produced by normotensives followed by lesser amounts of $PGF_{2\alpha}$ and PGE_2. 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were produced in similar amounts by the smooth muscle layer of GH rats with less PGE_2 formed. This difference in the profile of PG production between normotensive and GH rats was a significant ($P < 0.001$) decrease (Figure 4.3.1) in the quantity of 6-keto-$PGF_{1\alpha}$ produced and a significant ($P < 0.01$) increase in the production of $PGF_{2\alpha}$ by the smooth muscle layer of GH rats. There was no difference in PGE_2 production.

Figure 4.3.2 shows endothelial PG production expressed as ng of PG per 2.5 cm length of aorta. 6-keto-$PGF_{1\alpha}$ was the PG produced in greatest amounts by the endothelium of normotensives with lesser amounts of $PGF_{2\alpha}$ and PGE_2. In

Figure 4.3.2. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by an endothelial cell suspension from a 2.5cm length of aorta from normotensive and New Zealand genetically hypertensive male rats.



endothelial cells from the aorta of GH rats, the descending order of production was 6-keto-$PGF_{1\alpha}$ > $PGF_{2\alpha}$ > PGE_2. There was no difference in 6-keto-$PGF_{1\alpha}$ production between the two groups but there was an increased production of $PGF_{2\alpha}$ ($P < 0.01$) and a decreased production of PGE_2 ($P < 0.05$) by the endothelium from GH rats.

When the results were expressed as total PG production per 2.5 cm length of aorta, 6-keto-$PGF_{1\alpha}$ was the major PG produced by smooth muscle homogenates and by endothelial cell suspensions, whereas $PGF_{2\alpha}$ was the major PG produced by homogenates of adventitia. The total amounts of 6-keto-$PGF_{1\alpha}$ synthesised by the separate layers were in the order of smooth muscle > adventitia > endothelial cell layer for normotensive rats, and smooth muscle > adventitia = endothelial cell layer for GH male rats. The total amounts of $PGF_{2\alpha}$ and PGE_2 synthesised were higher by smooth muscle and adventitia than by endothelial cells of both groups of rats. However, as the endothelial cells constitute about 2% of the total tissue of the aorta, the capacity for synthesising 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ is 3 to 30-fold higher in the endothelial cells than in smooth muscle or adventitia.

The amounts of 6-keto-$PGF_{1\alpha}$ produced by homogenates of smooth muscle and adventitia were significantly lower ($P < 0.05$) in hypertensive compared to normotensive rats but the amounts of 6-keto-$PGF_{1\alpha}$ synthesised by the endothelial cells were similar between the two groups. $PGF_{2\alpha}$ synthesis was significantly higher ($P < 0.05$) by

Table 4.3.1.

Mean (± s.e. mean, n = 6) amounts of prostaglandins (PGs) formed by endothelial cell suspensions (Endo), and by homogenates of smooth muscle (SM) and adventitia (Adv) from the aorta of normotensive and hypertensive, male and female rats.

	6-keto-$PGF_{1\alpha}$			$PGF_{2\alpha}$			PGE_2		
	Endo	SM	Adv	Endo	SM	Adv	Endo	SM	Adv
	ng PG 2.5 cm length of aorta $^{-1}$								
Male:									
Normotensive	9.5 ± 1.7	52.8 ± 3.7	30.6 ± 3.1	2.2 ± 0.3	7.7 ± 1.5	44.7 ± 3.2	4.4 ± 0.5	15.8 ± 1.9	26.2 ± 3.4
Hypertensive	8.5 ± 0.7	31.8 ± 3.1^{+}	8.1 ± 1.2^{+}	6.0 ± 0.4^{+}	24.2 ± 3.8+	17.6 ± 4.2^{+}	2.8 ± 0.2^{+}	12.8 ± 2.6	11.0 ± 1.1^{+}
Female									
Normotensive	8.3 ± 2.0	74.1 ± 19.9	12.05 ± 1.1*	5.9 ± 1.7	40.0 ± 5.7*	36.6 ± 3.7	3.1 ± 3.7	23.0 ± 1.9*	21.0 ± 2.1
Hypertensive	2.4 ± 0.6*+	27.9 ± 2.8^{+}	6.8 ± 0.8^{+}	1.6 ± 0.2+*	17.4 ± 2.8^{+}	17.0 ± 2.2^{+}	3.9 ± 0.5	24.4 ± 2.9*	25.2 ± 1.9*

the smooth muscle and endothelial cell layers, and was significantly lower ($P < 0.05$) by adventitia of hypertensive compared to normotensive male rats. PGE_2 synthesis was significantly lower ($P < 0.05$) by the endothelial cell layer and adventitia, but was similar in smooth muscle of GH compared to normotensive male rats.

Significant differences in the production of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 by smooth muscle were present whether the results were expressed on a unit weight or a unit length basis. However, significant differences in the amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 produced by adventitia of normotensive and GH male rats disappear when the results are expressed on a unit weight as opposed to a unit length basis. Obviously,the amount of adventitia in the aorta is greater in normotensive compared to GH rats. Also, although both groups of rats were the same age, the body weight of the normotensive rats was slightly greater than that of the hypertensive rats. However, correcting the results for differences in body weight had no effect on the significant differences observed.

4.3.4 Conclusions

Arterial wall thickening in hypertension has been observed previously in rabbits and is the result of both hyperplasia and hypertrophy of the vascular smooth muscle (Bevan, 1976; Bevan, Eggena, Hume, Van Martheus and Bevan, 1980). Mulvany, Aalkjaer and Christensen, (1980)

have found a correlated increase in blood pressure and smooth muscle layer thickness in 12- and 24-week old SHR compared to normotensives of the same age, and Hume (1980) has demonstrated in the rabbit that protein synthesis and cell division can be induced in the vessel wall solely by an increase in wall tension. The increased protein content of blood vessels from hypertensive rats has been well documented. Folkow, (1978) has reviewed the evidence that the increased peripheral resistance of hypertension can be accounted for by an increased wall thickness; an altered vessel lumen to wall ratio producing the increased microvascular resistance response.

The finding that homogenates of smooth muscle have a considerable capacity to synthesise PGs is of interest since it is generally assumed that the endothelial cell layer is the only part of the blood vessel which synthesises PGs to any significant extent. Although this study was measuring PG synthesis in homogenates of smooth muscle incubated with exogenous AA, there is some evidence which suggests that PGI_2 synthesis by the smooth muscle layer may be important *in vivo*. Cultured smooth muscle cells from pig aorta have been shown to produce mainly PGE_2 and $PGF_{2\alpha}$ (Ager et al., 1982; Siess, et al., 1981; Ody et al., 1982 and 1983) but the possible importance of the ability of smooth muscle to produce PGI_2 can be appreciated from studies where the aortic endothelial cell layer was removed by balloon

catheterisation. Immediately after de-endothelisation the aorta was covered with adherent platelets, but, after several days only a few adherent platelets remained on the luminal surface. This decrease in the number of adherent platelets correlated with an increase in the production of PGI_2, from non-detectable levels to levels found in normal endothelium (Eldor et al. 1981; Tschopp and Baumgartner, 1981).

Interestingly, the smooth muscle and endothelial cell layers of hypertensive males produced significantly more $PGF_{2\alpha}$ than the same layers from normotensive male rats. However, the endothelial cell layer from GH rats also produced significantly lower amounts of PGE_2 than normotensives therefore, it is not possible to discount that the increased amount of $PGF_{2\alpha}$ measured was due to increased activity of 9-PGE-KR in GH rats, thus increasing conversion of PGE_2 to $PGF_{2\alpha}$. There was a concomitant decrease in the production of 6-keto-$PGF_{1\alpha}$ in the smooth muscle, but not the endothelial cell layer from GH rats. This increase in blood vessel $PGF_{2\alpha}$, but not the decrease in 6-keto-$PGF_{1\alpha}$ has already been observed in homogenates of whole aorta from hypertensive rats (see section 4.1).

SECTION 4.4

Measurement of unstimulated PG release from the isolated perfused aorta.

4.4.1 Introduction

Measurement of PG production from exogenous arachidonic acid in homogenates gives an indication of the PG synthetase enzymes present in the tissue and of the synthetic capacity for the different PGs, but it does not necessarily reflect PG output in the whole animal. The perfusion of an intact vessel and collection of the perfusion fluid, with subsequent measurement of the PGs present, probably gives a more accurate profile of PG release *in vivo*.

The experiments described in this sub-section, therefore, were carried out to determine the profile of PGs released from the isolated perfused aorta of normotensive and GH male rats.

4.4.2 Methods

Male albino Wistar rats aged between 2 and 3 months and weighing 250-300g and GH rats of the same age, weighing 200-250g were used. The animals were housed as before.

Six animals from each group were killed and the thoracic aorta was cannulated. A 2.5 cm portion of aorta was

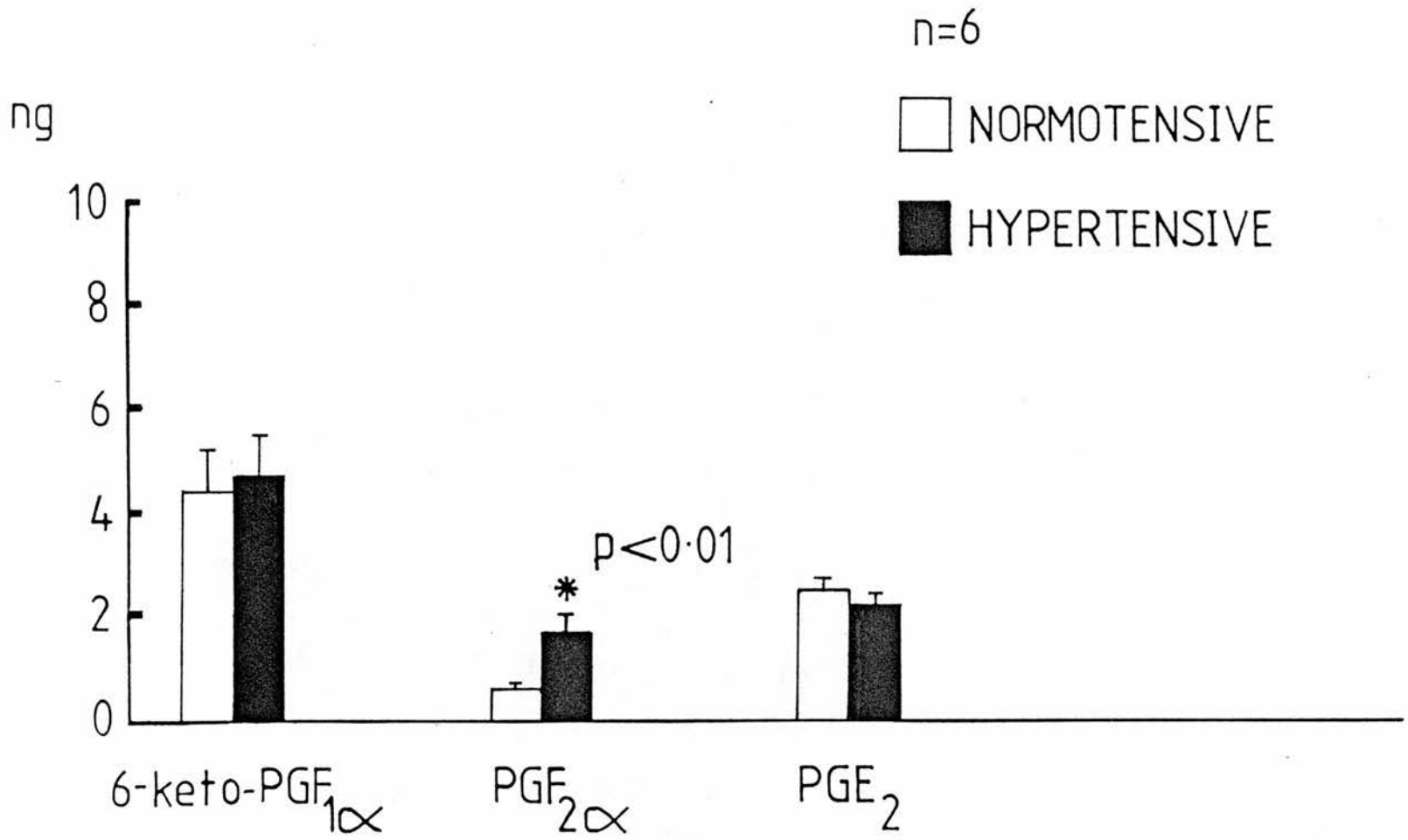
removed, was placed in a heated (37°C) chamber and perfused with oxygenated (95% O_2, 5% CO_2) Krebs solution at a rate of 4 ml/min. After 30 min perfusion to allow for equilibration of the tissue after the trauma of dissection, the perfusate was collected over a period of 30 min. The pH of the perfusate was taken to pH4 with HCl and was extracted twice with two volumes of ethyl acetate, which is the same extraction procedure as was described for homogenates in Section 2.4. The samples were stored in ethyl acetate until assayed by RIA for 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 content as detailed in Section 2.5.

Results for normotensive and GH male rats were compared using Student's t-test and significance was tested at the 5% level.

4.4.3 Results

Figure 4.4.1 shows the basal release of PGs during a 30 min period from the aortae of normotensive and GH male rats. 6-keto-$PGF_{1\alpha}$ was the major PG released by normotensives, followed by PGE_2 and a very small amount of $PGF_{2\alpha}$. In the aorta from GH rats 6-keto-$PGF_{1\alpha}$ was the main PG released with similar amounts of $PGF_{2\alpha}$ and PGE_2 measured. This was a significant increase ($P < 0.01$) in the amount of $PGF_{2\alpha}$ released from the perfused aorta of GH rats. There was no difference in the amounts of 6-keto-$PGF_{1\alpha}$ or PGE_2 released between the 2 groups.

Figure 4.4.1. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) released from a 2.5cm length of isolated, perfused aorta from normotensive and New Zealand genetically hypertensive male rats.



4.4.4 Conclusions

A recent study by Elliot and Adolfs, (1984) has shown that PGI_2 release by the isolated, perfused aorta is initially high but gradually declines to a stable level which is maintained for at least 180 min. Levels of 6-keto-$PGF_{1\alpha}$, measured by RIA, reflected the changes in biological activity monitored on the superfused rat stomach strip. Thus, the isolated perfused aorta appears to be a good model for investigating the profile of endogenous PG release.

In this study, a different profile of PG release from intact aorta compared to PG synthetic capacity of homogenates was observed. The amount of 6-keto-$PGF_{1\alpha}$ released was much greater than that of PGE_2, and the amount of $PGF_{2\alpha}$ released was much lower than the other 2 PGs. The ratio of 6-keto-$PGF_{1\alpha}$:$PGF_{2\alpha}$:PGE_2 was 1:0.3:0.2 for aortic homogenates compared with 1:0.1:0.6 for release from intact aorta in normotensive rats, and was 1:0.6:0.2 for aortic homogenates compared with 1:0.4:0.4 for release from intact aorta of GH rats. The release of $PGF_{2\alpha}$ from the aorta of GH rats was markedly increased (3-fold) compared to normotensives and this was possibly a reflection of the increased synthetic capacity of the aortic homogenates from hypertensives to produce $PGF_{2\alpha}$.

Similar to the observations made using homogenates of aorta, 6-keto-$PGF_{1\alpha}$ release from the intact aorta was not different between normotensive and GH rats. This is not

in agreement with the findings of Okuma et al., 1980 and Pace-Asciak et al. (1978) using aortic rings from SHR, or of Botha et al., 1980 using aortic strips from the GH strain of hypertensive rats. However, the spontaneous release of PGI_2 from aortic rings or strips is probably due to the metabolism of arachidonic acid released by PLA_2 from the damaged cells, and therefore may reflect the ability of the damaged tissue to synthesise PGI_2 rather than the true basal rate of synthesis.

SECTION 4.5

Measurement of stimulated PG release from the isolated, perfused mesenteric arterial bed of normotensive and genetically hypertensive male rats.

4.5.1 Introduction

Although the aorta is generally the blood vessel most often studied with regard to vascular PG synthesis it is not necessarily typical of the vascular system, and in particular of the small resistance vessels such as the arterioles. The isolated, pefused mesenteric arterial bed preparation, if dissected carefully, retains the majority of its small resistance vessels and can be used to investigate the actions of natural or pharmacological compounds on a resistance bed. This preparation then, can be used to study the PG-releasing abilities of some of these compounds.

Angiotensin II-(AII)-stimulated PG release

The potent vasoactive hormone, AII has also been studied in relation to its ability to cause the release of PGs. AI and AII caused the release of a PGI_2-like substance (measured on a platelet bioassay) from the rabbit mesenteric artery (Grodzinska et al., 1980) and the release of a PGE_2-like substance from the same rabbit preparation (Blumberg et al., 1977). Saralasin, an AII analogue and a competitive inhibitor, prevented the AII-induced release of PGE_2, but did not block PGE_2 release by bradykinin. Similarly, Grodzinska et al. (1980) found that saralasin inhibited the AII-stimulated, but not the bradykinin-stimulated release of PGI_2. Captopril, a converting enzyme inhibitor abolished the release of PGE_2 and PGI_2 induced by AI, but had no effect on AII-induced PG release. These results suggest that the release of PGs stimulated by AII is mediated via a specific AII-receptor. Exogenous PGE_2 and PGI_2 reduce the vasoconstrictor response to AII in rabbit mesenteric artery (Malik et al., 1976) and in rabbit hindquarters vascular bed (Gottlieb et al., 1980).

Although the synthetic capacity of blood vessel homogenates for PG synthesis and the basal release of PGs from blood vessel pieces in the hypertensive state have been studied quite extensively, there has been very little information published on the stimulated release of PGs from the vessels of hypertensive animals. Basal PGI_2 release from the mesenteric vascular bed of hypertensive rats was found to be higher than in normotensives but nerve stimulation failed to increase PGI_2 and PGE_2

release from the mesenteric bed of hypertensive compared to normotensive rats (Pipili and Poyser, 1982). In the present study, the effects of NA and AII on PG output from the mesenteric vascular beds of GH and normotensive male rats has been investigated.

Noradrenaline-(NA)-stimulated PG release

An infusion of NA into the mesenteric vascular preparation of the rabbit produced a rise in perfusion pressure and the release of a PGE-like material. Indomethacin prevented, but AA augmented this NA-stimulated release of a PGE-like material (Grodzinska et al., 1976). Simmet et al. (1980) however, using the same preparation in the rabbit found that NA did not produce any detectable PG release. A high K+ concentration was the stimulus which induced contractions of the vascular bed and PGE release. Using the rat mesenteric vascular bed Coupar et al. (1980) found that indomethacin inhibited the vasoconstrictor effect of NA, and in contrast to the findings in the rabbit, observed that the pressor responses were actually restored by exogenous PGE_2, or even more potently by its analogue 12,S-15 methyl PGE_2 methyl ester. Exogenous PGI_2 and PGE_2 reduced vasoconstrictor responses to NA and nerve stimulation in rabbit mesenteric arteries (Malik et al., 1976) and in the rabbit hindquarters vascular bed (Gottlieb et al., 1980). PGE_2 and PGI_2 are also released from the mesenteric artery of rats and rabbits following nerve stimulation, (Horton et al., 1980; Pipili and

Poyser, 1981) but it is PGE_2 and not PGI_2 which reduces the output of NA from the rabbit mesenteric artery during nerve stimulation (Armstrong and Thirsk, 1979).

4.5.2 Methods

Normotensive albino Wistar male rats and male GH rats of 2-3 months of age and weighing 250-300g and 200-250g respectively were used. The mesenteric arterial bed was prepared according to McGregor (1965) with some modifications. Briefly, the superior mesenteric artery was cannulated about 2 cm distal to the abdominal aorta. The entire mesentery was carefully dissected away from the gut and the vascular bed was removed to a heated (37°C) chamber where it was perfused at 4 ml/min with oxygenated (95% O_2, 5% CO_2) McEwen's solution. The vascular bed was perfused for 30 min to allow for recovery of the tissue after the trauma of surgery.

NA and AII (0.1 μg and 1.0 μg) were given as a bolus injection in a random order. Samples were collected at 1 min intervals for the 2 min before stimulation, for the 4 min immediately after stimulation and for a further two periods, 8 min and 12 min after stimulation. Pressor responses were recorded on a Polygraph recorder via a Statham pressure transducer. 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 content in the perfusion fluid were measured by RIA, without extraction. It has been shown previously that the PG content of perfusates does not differ significantly between extracted and non-extracted samples

(Pipili and Poyser, 1981). The effects of NA and AII on PG release were tested for significance by comparing the 1 min period prior to stimulation with the two 1 min periods post-stimulation using Student's t-test for paired data. Significance was tested at the 5% level.

4.5.3 Results

6-Keto-$PGF_{1\alpha}$ was the major PG released by the mesenteric vascular bed of normotensive rats, with considerably less PGE_2 and $PGF_{2\alpha}$ being released. The amounts of $PGF_{2\alpha}$ released into the perfusate during the 1 min collection period were below the detection limit of the assay (< 30 ng/min). In the mesenteric vascular bed of hypertensive rats, 6-keto-$PGF_{1\alpha}$ was also the major PG released followed by lower amounts of PGE_2 and $PGF_{2\alpha}$.

The release of all 3 PGs declined over the duration of the experiment (60 min) in both groups of rats, as shown in Table 4.5.1.

Table 4.5.1

The release of prostaglandins (PGs) from the isolated, perfused mesenteric arterial bed of normotensive and hypertensive male rats mean+/-s.e.m., n=4; ND- < 30 ng/min).

	Normotensive		Hypertensive	
PG ng/min	START	END	START	END
6-Keto-$PGF_{1\alpha}$	1.30+/-0.22	0.98+/-0.22	1.94+/-0.22	1.09+/-0.19
PGE_2	0.54+/-0.10	0.36+/-0.03	0.80+/-0.27	0.48 (2 ND)
$PGF_{2\alpha}$	ND	ND	0.44+/-0.06	ND

4.5.3.1 AII-stimulated PG release

Both the low (0.1 μg) and high (1.0 μg) dose of AII caused a significant (P <0.05) increase in the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed of male normotensives (Figure 4.5.1). Neither dose of AII produced any stimulation of $PGF_{2\alpha}$ release above non-detectable levels. Although there was some stimulation of PGE_2 release above base-line levels at the 0.1 μg dose of AII, this was not significant. However, the higher dose of 1.0 μg AII produced a significant (P < 0.01) increase in the release of PGE_2 from the mesenteric vascular bed of male normotensives (Tables 4.5.2 and 4.5.3). The bolus injections of AII had a pressor effect

Figure 4.5.1. Angiotensin II (AII)-stimulated release of prostaglandin (PG)I_2, measured as 6-keto-PGF$_{1\alpha}$ from the isolated, perfused mesenteric arterial bed of 4 normotensive - B, and 4 New Zealand genetically hypertensive male rats -A. * compared to value immediately preceding stimulation.

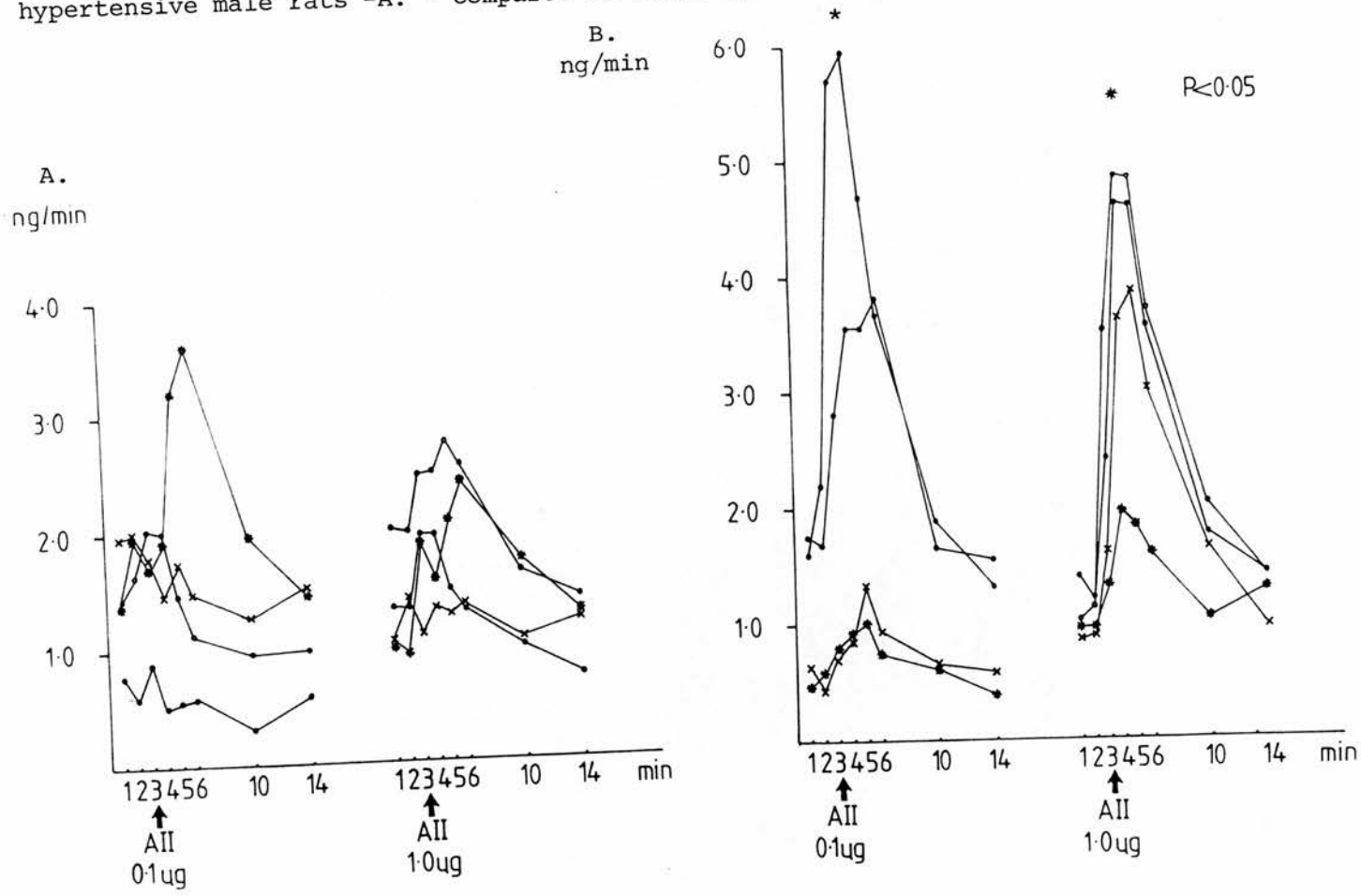


Table 4.5.2.

Angiotensin II(AII)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive male rats (ND-< 20 pg/min)

	$PGF_{2\alpha}$ release $ngmin^{-1}$															
			0.1 µg A II ↓								1.0 µg A II ↓					
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
NORMOTENSIVE MALES	ND →								ND →							
	ND →								ND →							
	ND →								ND →							
	ND →								ND →							
HYPERTENSIVE MALES	ND →								0.48	0.45	0.54	0.53	0.34	0.27	ND	ND
	ND →								ND →							
	ND →								ND	ND	ND	0.56	0.59	0.37	0.34	0.34
	0.61	0.42	0.32	ND →					0.26	0.80	0.66	0.50	0.75	0.35	0.37	ND

Table 4.5.3.

Angiotensin II(AII)-stimulated release of prostaglandin (PG)E_2 from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive male rats (ND-< 20 pg/min)

PGE_2 release $ngmin^{-1}$

			0.1 µg AII ↓								1.0 µg AII ↓						
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14	
	0.50	0.59	0.22	0.22	0.46	0.37	0.37	ND	0.26	0.38	1.2	1.02	0.77	0.82	0.69	ND	
NORMOTENSIVE	0.34	0.43	0.70	ND	ND	0.43	0.85	0.60	ND	0.40	0.77	0.77	0.60	0.69	0.37	0.40	
MALES	0.67	0.67	1.26	0.29	0.60	0.54	0.77	0.64	0.52	0.58	1.32	1.33	0.64	0.72	0.70	0.78	
	0.80	0.82	0.74	1.33	0.94	0.80	ND	ND	0.81	0.66	0.46	1.08	0.98	0.50	0.90	1.06	
	1.20	1.16	1.09	1.04	0.83	1.16	1.01	0.53	0.48	0.45	0.68	0.60	0.67	1.06	ND	ND	
HYPERTENSIVE	ND	→							ND	→							
MALES	ND	0.78	0.50	0.25	0.82	0.37	0.85	ND	ND	→							
	ND	→							0.24	0.32	0.52	0.54	0.21	ND	ND	ND	

on the vascular bed producing a 28.8 +/- 9.8 % increase in the basal perfusion pressure (42.5 +/- 3.7 mmHg) at 0.1 μg AII and an increase of 57.2 +/- 7.7 % at the 1.0 μg dose of AII. This was a significant ($P < 0.01$) increase in perfusion pressure at the higher dose of AII.

In the mesenteric vascular bed from male hypertensives both the 0.1 μg and 1.0 μg doses of AII failed to elicit a significant increase in the basal release of 6-keto-$PGF_{1\alpha}$. Similarly, there was no significant change in the release of $PGF_{2\alpha}$ and PGE_2 at either dose of AII from the mesenteric bed of male hypertensives. AII also had a pressor effect on the vascular bed of hypertensives with the 0.1 μg dose causing a 58.0 +/- 20.2% increase and the 1.0 μg dose giving a 136 +/- 30.3% increase in basal perfusion pressure (37.5 +/- 2.5 mmHg). The increase in perfusion pressure was significant ($P < 0.05$) at the higher dose of AII. There was a significantly greater ($P < 0.05$) pressor response to the 1.0 μg dose of AII in the mesenteric vascular bed of hypertensives compared to normotensives.

4.5.3.2 NA-stimulated PG release

Both doses of NA produced a significant increase (0.1 μg NA; $P < 0.05$, 1.0 μg NA; $P < 0.01$) in the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed of male normotensives (Figure 4.5.2). NA did not appear to affect the release of $PGF_{2\alpha}$ as the amounts of this PG remained below the detection limit of the assay.

Figure 4.5.2. Noradrenaline (NA)-stimulated release of prostaglandin (PG)I_2, measured as 6-keto-$PGF_{1\alpha}$ from the isolated, perfused mesenteric arterial bed of 4 normotensive - B, and 4 New Zealand genetically hypertensive male rats - A. * compared to value immediately preceding stimulation.

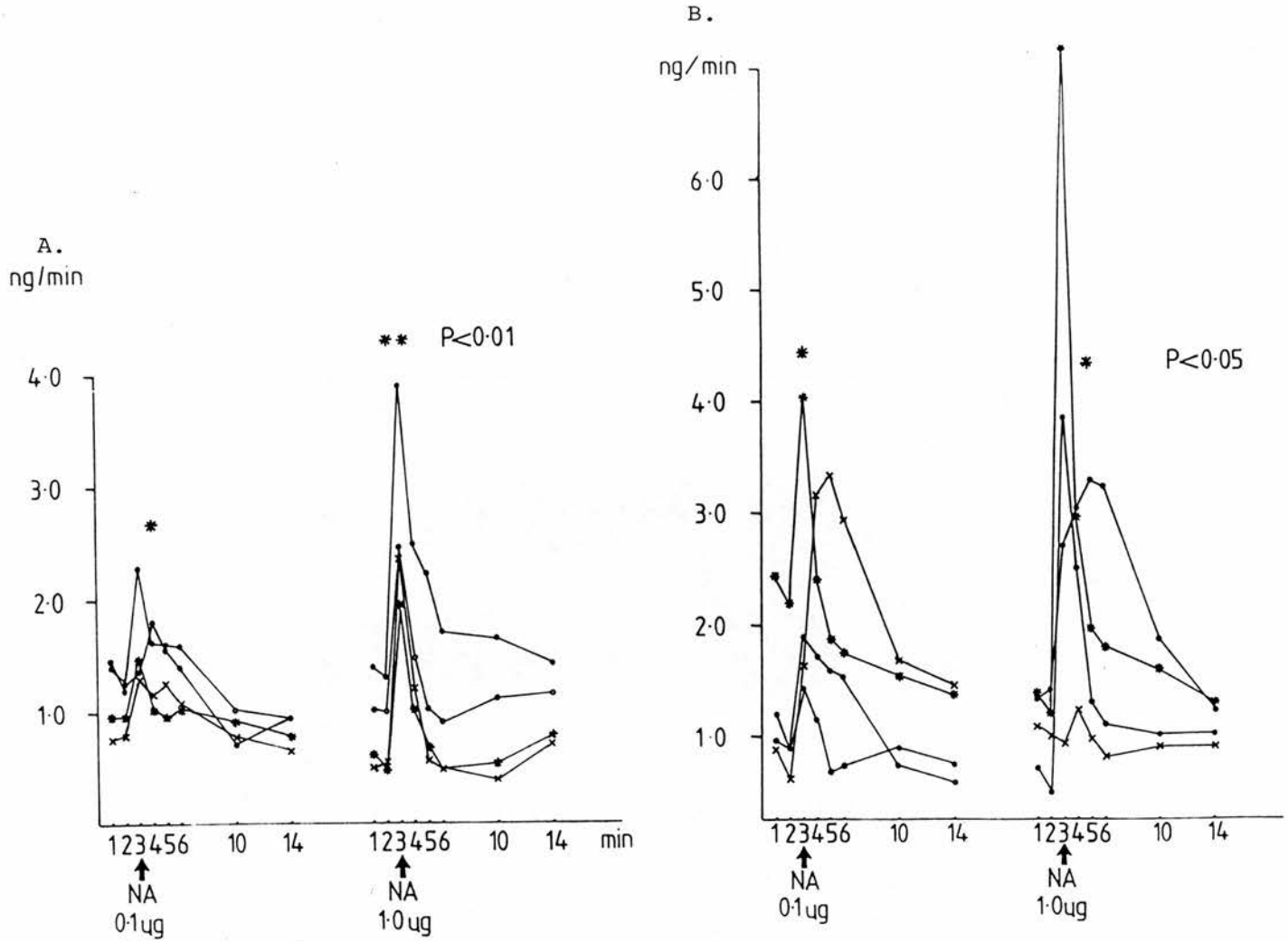


Table 4.5.4.

Noradrenaline (NA)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive male rats (ND-< 20 pg/min)

$PGF2_{\alpha}$ release $ngmin^{-1}$

			0.1 µg NA ↓								1.0 µg NA ↓						
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14	
	ND	→							ND	→							
NORMOTENSIVE	ND	→							ND	→							
MALES	ND	→							ND	→							
	ND	→							ND	→							
	ND	→							ND	→							
HYPERTENSIVE	0.50	0.44	0.92	0.50	0.30	0.29	ND	ND	ND	ND	1.08	0.52	0.24	ND	ND	ND	
MALES	0.16	0.42	0.34	0.73	0.64	0.19	0.21	ND	ND	0.50	0.93	1.18	0.83	0.92	1.71	0.32	
	0.21	0.21	0.21	0.42	0.40	0.27			ND	0.29	0.55	0.24	ND	ND	ND	ND	

Table 4.5.5.

Noradrenaline(NA)-stimulated release of prostaglandin (PG)E_2 from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive male rats (ND-< 20 pg/min)

PGE_2 release $ngmin^{-1}$

	0.1 µg NA ↓								1.0 µg NA ↓							
	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
	ND	ND	ND	0.61	0.52	ND	ND	ND	ND	ND	0.54	0.46	0.60	0.64	0.24	0.30
NORMOTENSIVE	ND	ND	ND	0.90	0.67	0.41	0.90	0.82	0.64	0.30	ND	0.86	0.55	0.46	0.66	ND
MALES	0.52	ND	1.17	0.44	0.50	0.78	ND	ND	0.60	0.94	0.89	1.06	0.24	0.22	0.19	0.37
	0.96	ND	ND	0.78	0.53	0.72	0.37	ND	0.34	0.53	0.24	0.51	0.62	ND	ND	ND
	0.64	0.66	0.64	1.24	0.58	0.82	0.50	0.46	0.20	0.22	0.60	0.53	ND	ND	0.24	ND
HYPERTENSIVE	0.44	0.85	1.05	1.14	0.74	0.64	0.46	0.63	0.38	0.43	0.59	0.66	0.54	0.76	0.75	0.55
MALES	ND	ND	0.36	0.43	ND	ND	ND	ND	1.32	0.8	1.36	0.58	0.78	0.74	0.24	0.32
	ND	→							0.40	0.37	0.21	0.40	0.34	0.60	0.50	ND

Similarly, NA at either dose, had no effect on the release of PGE_2 from the mesenteric bed of male normotensives (Tables 4.5.4 and 4.5.5). NA also had a pressor effect on the mesenteric vascular bed. 0.1 μg produced a 63.2 +/- 34.7% increase, and 1.0 μg a 174.0 +/- 4.4% increase in perfusion pressure. The higher dose of NA produced a significant ($P < 0.01$) increase in perfusion pressure.

In the mesenteric vascular bed of male hypertensives, NA at both the 0.1 μg and 1.0 μg doses produced a significant ($P < 0.05$) increase in the basal release of 6-keto-$PGF_{1\alpha}$. There was no stimulation of $PGF_{2\alpha}$ release after the low dose of NA and although there was some stimulation at the higher dose, this was not significant. Both doses of NA failed to elicit any increase in PGE_2 release from the mesenteric bed of male GH rats. NA at the 0.1 μg dose caused a 153.0 +/- 43.5% increase in perfusion pressure. The higher dose of NA gave a 261 +/- 33.5% increase in perfusion pressure. This was a significant increase in perfusion pressure at both the low ($P < 0.05$) and high ($P < 0.01$) doses of NA.

4.5.4 Conclusions

To summarise briefly by comparing the responses of the mesenteric beds from normotensives and hypertensives, AII (1.0 μg) elicited an increase in the release of 6-keto-$PGF_{1\alpha}$ and PGE_2 from the mesenteric bed of normotensive but not from GH male rats. The release of

$PGF_{2\alpha}$ was not affected by AII in either the normotensive or hypertensive group. AII (1.0 μg) produced a significantly ($P < 0.05$) greater increase in perfusion pressure in the mesenteric bed from hypertensive compared to normotensive male rats.

Both doses of NA gave an increase in the release of 6-keto-$PGF_{1\alpha}$, without affecting the release of $PGF_{2\alpha}$ and PGE_2 in normotensive and hypertensive males. NA (1.0 μg) also produced a significantly greater increase in mesenteric perfusion pressure in GH compared to normotensive rats.

SECTION 4.6

Measurement of PG production in the separate layers of aorta from normotensive and GH female rats.

4.6.1 Introduction

The conflicting effects of the sex steroids on thrombosis and vascular PGI_2 production have been discussed in Section 3.6. Testosterone enhanced thrombus formation in male and female rats whereas oestradiol reduced thrombus size in male rats, but not in female rats (Uzonova et al., 1976). However, a greater release of PGI_2 from aortic rings of male compared to females was found (Maggi et al., 1980, Pomerantz et al., 1980). Oestrogen was found by various authors to conversely either inhibit or stimulate PGI_2 release from cultured rat aortic smooth muscle cells or rat aortic rings.

The experiments described in Section 2, established that there are significant differences in the PG synthetic capacity of homogenates of different blood vessels from male compared to female rats. However, PG production by homogenates of aorta did not differ between day 1 and day 4 of the oestrous cycle and PG production was not affected by short-term OVX and oestrogen and progesterone treatment.

As there is such a paucity of information in the literature on the production or endogenous release of PGs

in the female, further experiments were carried out in the female rat to characterise the profiles of vascular PG production and release and to contrast these findings with those observed in the male rat.

4.6.2 Methods

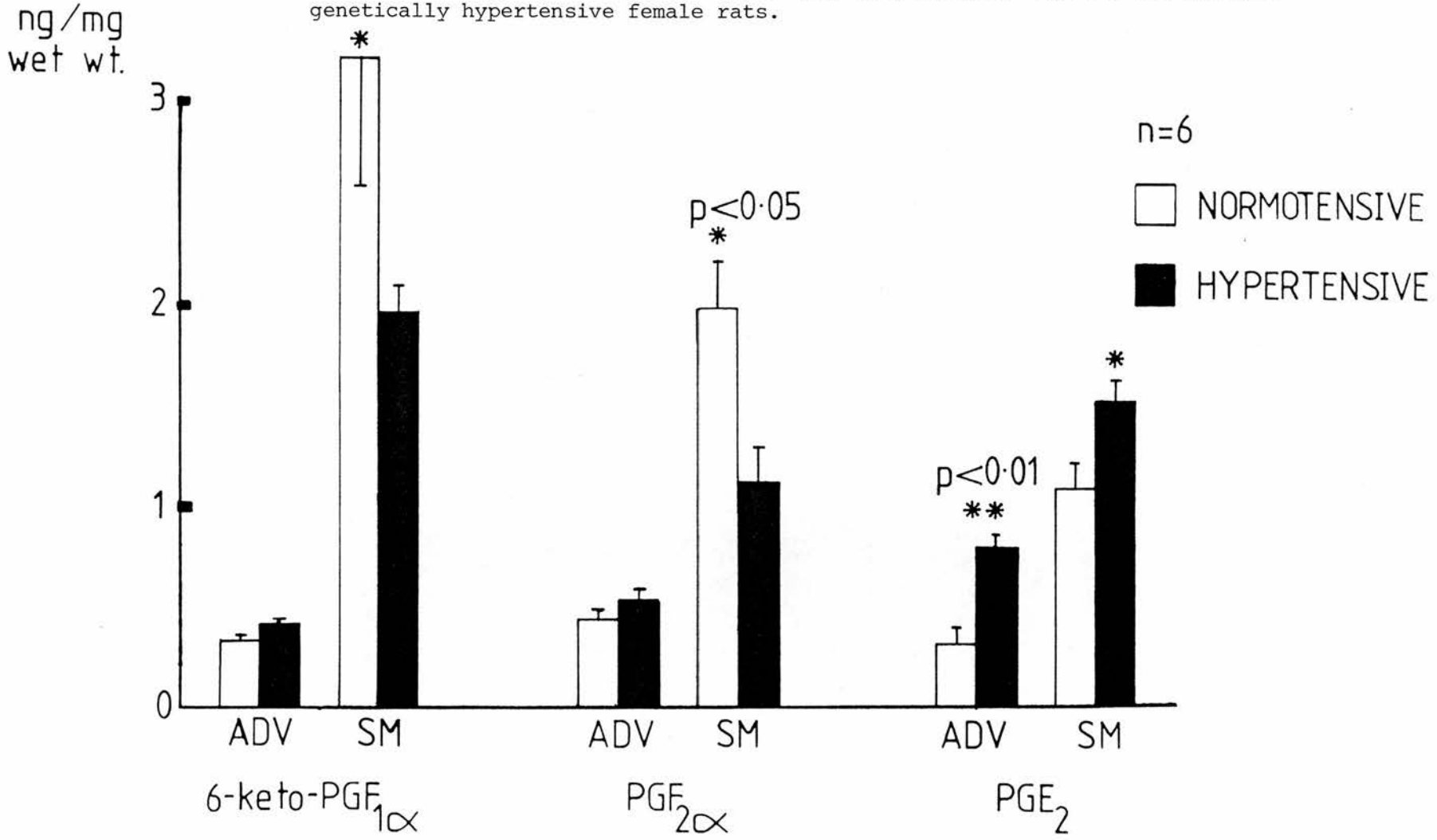
Female albino Wistar rats aged 2-3 months and weighing 200-250g and female GH rats of the same age weighing 180-200 g were used. Animals were housed and fed as before. Vaginal smears were taken daily and after two consecutive 4-day oestrous cycles had been established, the animals were killed at 10:00h on pro-oestrus (day 4).

The aortae were removed and the three different layers were separated, homogenised and incubated as described in Section 2.4. Samples in ethyl acetate were stored at -20°C until assayed by RIA for PG content as detailed in Section 2.5. The results from normotensive and GH female rats were compared using Student's t-test and significance was tested at the 5% level.

4.6.3 Results and Conclusions

Figure 4.6.1 compares PG production by homogenates of the adventitia and smooth muscle layers of aorta from normotensive GH, day 4 female rats expressed as ng per mg wet weight. The adventitia from normotensives produced similar amounts of the 3 PGs. PGE_2 was the PG produced in greatest amounts by the adventitia of GH rats with

Figure 4.6.1. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by homogenates of adventitia (ADV) and smooth muscle (SM) from normotensive and New Zealand genetically hypertensive female rats.



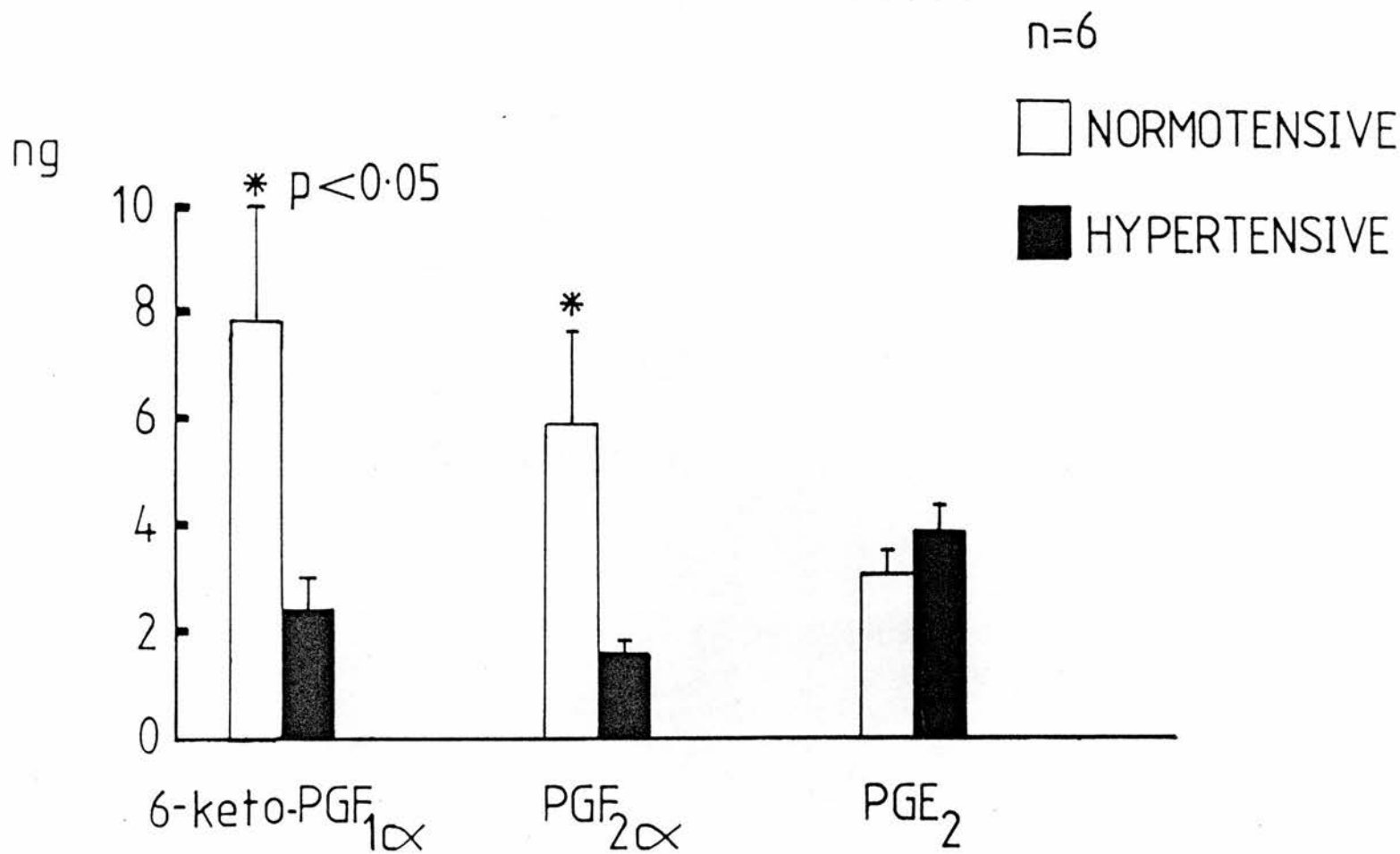
lower amounts of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$. PGE_2 production by the adventitia was significantly greater ($P < 0.01$) in GH than in normotensive female rats. In the smooth muscle layer 6-keto-$PGF_{1\alpha}$ was the major PG produced by normotensives followed by $PGF_{2\alpha}$ and PGE_2. In GH rats 6-keto-$PGF_{1\alpha}$ was also the main PG produced but PGE_2 production was greater than that of $PGF_{2\alpha}$. The differences between the 2 groups were a decreased production of both 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ ($P < 0.05$) and an increased production of PGE_2 ($P < 0.01$) by the smooth muscle layer of GH rats compared to normotensive female rats.

Figure 4.6.2 shows endothelial PG production expressed as ng of PG per 2.5 cm length of aorta. The endothelial cell layer from normotensives produced mainly 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ with less PGE_2. However, in GH rats PGE_2 was the main PG produced with similar amounts of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$. There were significant decreases ($P < 0.05$) in the production of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ by GH females compared with normotensive female rats.

Table 4.3.1 shows a comparison of the mean PG production by homogenates of adventitia and smooth muscle and by an endothelial cell suspension, expressed per unit length, from the aorta of day 4 female normotensive and GH rats.

6-Keto-$PGF_{1\alpha}$ was the major PG produced by smooth muscle homogenates of normotensive and GH female rats.

Figure 4.6.2. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by an endothelial cell suspension from a 2.5cm length of aorta from normotensive and New Zealand genetically hypertensive female rats.



6-Keto-$PGF_{1\alpha}$ was also the major PG produced by the endothelial cell suspension of normotensives, but $PGF_{2\alpha}$ was the major PG produced by this layer of GH female rats. $PGF_{2\alpha}$ was the major PG produced by the adventitia of both groups of female rats, followed by PGE_2 and 6-keto-$PGF_{1\alpha}$. The total amounts of 6-keto-$PGF_{1\alpha}$ synthesised by the separate layers were in the order of smooth muscle > adventitia > endothelial cell layer for normotensive and hypertensive female rats. However, as discussed previously in relation to male rats, as the endothelial cells constitute a very small percentage (approx. 2%) of the total aortic tissue, the capacity for synthesising the 3 PGs is 3- to 30-fold higher in the endothelial cells than in the smooth muscle or adventitia.

The amounts of 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$ synthesised by adventitia, smooth muscle and endothelial cells were significantly lower ($P < 0.05$) in hypertensive compared to normotensive female rats. There was no difference in PGE_2 production by the three layers in normotensive compared to GH rats.

4.6.4. Comparison of the amounts of PGs produced by separate layers of aorta of male and female, normotensive and hypertensive rats.

PG production per mg wet weight

There was no significant difference in the production of

any of the 3 PGs by homogenates of adventitia of male normotensives compared to female normotensives. $PGF_{2\alpha}$ production by smooth muscle homogenates of females was significantly ($P < 0.001$) greater than that by homogenates of males, whereas 6-keto-$PGF_{1\alpha}$ and PGE_2 production did not differ between the 2 groups. 6-keto-$PGF_{1\alpha}$ and PGE_2 production by the endothelial cell suspension did not show any sex difference and, although $PGF_{2\alpha}$ production was greater by the endothelium of females than males, this difference was not significant (Student's t-test).

There was no significant sex difference in the production of the 3 PGs by the adventitia of aortae from hypertensive rats. The production of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were not significantly different by homogenates of aortic smooth muscle layer of male compared to female hypertensive rats. Smooth muscle layer PGE_2 production, however, was significantly ($P < 0.05$) greater in hypertensive females compared to hypertensive males. 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ productions were greater ($P < 0.001$) by the endothelial cell suspension from GH males than from GH females. PGE_2 production by the endothelial cells was not significantly different between the two groups.

PG production per unit length

Table 4.6.1 shows a comparison of the mean production of PGs by the separate layers of aorta of normotensive and GH male rats compared to normotensive and GH female rats.

6-Keto-$PGF_{1\alpha}$ synthesis was significantly lower ($P < 0.05$) by the adventitia of female compared to male normotensive rats, but there was no difference in 6-keto-$PGF_{1\alpha}$ synthesis by the smooth muscle or endothelial cell layers. The amounts of $PGF_{2\alpha}$ and PGE_2 produced by homogenates of smooth muscle, but not by adventitia or endothelial cells, were significantly higher ($P < 0.05$) in female than in male normotensive rats.

6-Keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ synthesis were significantly lower ($P < 0.05$) by the endothelial cells of female hypertensive compared to male hypertensive rats, but there was no difference in 6-keto-$PGF_{1\alpha}$ or $PGF_{2\alpha}$ synthesis by the smooth muscle or adventitial layers. PGE_2 synthesis was significantly ($P < 0.05$) greater by the smooth muscle and adventitia, but not by the endothelial cells of female hypertensives compared to male hypertensives.

SECTION 4.7

Measurement of basal PG release from the isolated perfused aorta of normotensive and GH female rats.

4.7.1 Introduction

Tissue homogenates give an indication of the PG synthesising enzymes present in a particular tissue and of their capacity for generating PGs but they probably do

not provide an accurate representation of the amounts of PGs released *in vivo*. In this context, the reasons for using the isolated intact aorta for the measurement of basal release of PGs have been discussed previously with regard to male rats (Section 4.4) and these also apply to female rats.

Consequently, the profile of PG release from the isolated, perfused aorta of female normotensive and GH rats was determined.

4.7.2 Methods

Female albino Wistar rats and female GH rats aged 2-3 months and weighing 200-250 g and 180-200 g respectively were used. Consecutive 4-day oestrous cycles were established by taking vaginal smears daily and six animals from each group were killed on day 4 at 10:00h.

The thoracic aorta was cannulated and a 2.5 cm portion was carefully removed and placed in a heated (37°C) chamber. The isolated aorta was perfused at 4 ml/min with oxygenated (95% O_2, 5% CO_2) Krebs solution. After an equilibration period of 30 min, the perfusate was collected during the next 30 min period. The pH of the perfusate was taken to 4 with 1N HCl and the PGs were extracted twice with 2 volumes of ethyl acetate as described in section 2.4. The samples were stored in ethyl acetate until assayed by RIA for PG content as detailed in Section 2.5.

Results for normotensive and GH rats were compared using Student's t-test and significance was tested at the 5% level.

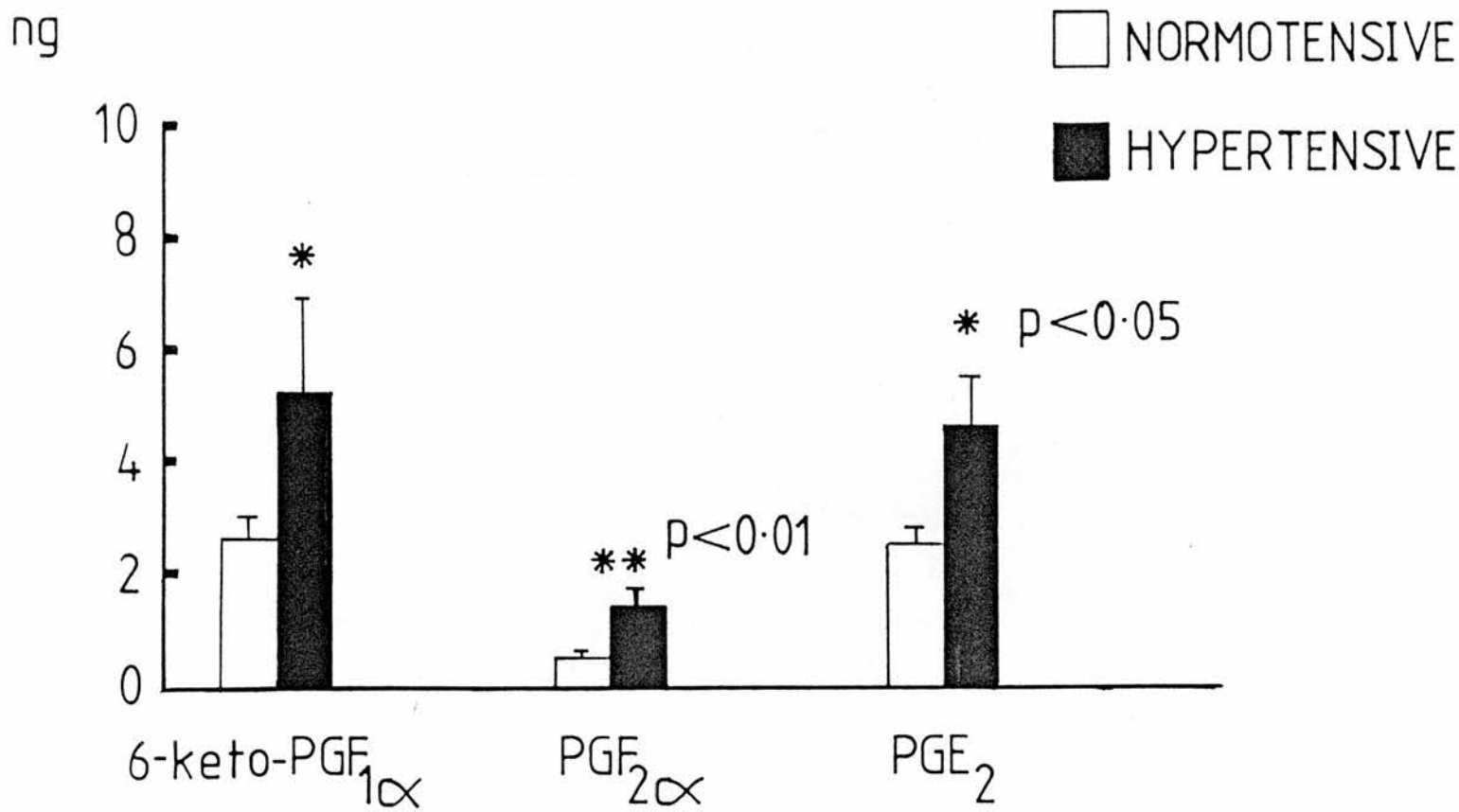
4.7.3 Results

As shown in Figure 4.7.1, 6-keto-$PGF_{1\alpha}$ and PGE_2 were the major PGs released from the aorta with considerably less $PGF_{2\alpha}$ measured. The perfused aorta of hypertensive females released similar amounts of 6-keto-$PGF_{1\alpha}$ and PGE_2 with less $PGF_{2\alpha}$. There was an increased release of 6-keto-$PGF_{1\alpha}$ ($P < 0.05$), PGE_2 ($P < 0.05$) and $PGF_{2\alpha}$ ($P < 0.01$) from the aorta of GH compared to normotensive female rats.

4.7.4 Conclusions

Unlike the homogenates of the separated layers of aorta and the endothelial cell layer, where 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ production were greater by normotensives than by hypertensives, the perfused aorta from hypertensives released greater amounts of all 3 PGs. This finding emphasises that the production of PGs measured in homogenates *in vitro* does not necessarily reflect PG output from the intact tissue *in vitro* . The latter parameter may be a better reflection of the *in vivo* situation.

Figure 4.7.1. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs)released from a 2.5cm length of isolated,perfused aorta from normotensive and New Zealand genetically hypertensive female rats.



4.7.5. Comparison of basal PG release from the aorta of male and female normotensive and hypertensive rats.

The basal release of 6-keto-$PGF_{1\alpha}$ was significantly ($P < 0.05$) greater from the isolated, perfused aorta of male compared to female normotensive rats, but there was no difference in the release of $PGF_{2\alpha}$ and PGE_2 between the sexes (Table 4.7.1).

6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ release from the aorta of GH rats was not significantly different when comparing male and female rats. The release of PGE_2 was significantly ($P < 0.05$) greater from the perfused aorta of female hypertensive compared to male hypertensive rats.

SECTION 4.8

Measurement of stimulated PG release from the isolated, perfused mesenteric arterial bed of normotensive and genetically hypertensive female rats.

4.8.1 Introduction

The isolated, perfused mesenteric arterial bed provides a vascular preparation which can be used to study the actions of pressor compounds on a resistance bed, and the possible PG-releasing properties of these compounds. This preparation was used to investigate the effects of NA and AII on PG release in normotensive and hypertensive female rats.

Table 4.7.1. Comparison of the basal output of prostaglandins (PGs), (mean +/-s.e.m.,n=6) from the isolated, perfused aorta of male and female, normotensive and hypertensive rats.

	PG output (ng/30 min/2.5cm length of aorta)			
	Normotensive		Hypertensive	
	Male	Female	Male	Female
6-Keto-$PGF_{1\alpha}$	4.4 +/- 0.8	2.6 +/- 0.4 *	4.7 +/- 0.8	5.2 +/- 1.7
$PGF_{2\alpha}$	0.6 +/- 0.07	0.5 +/- 0.05	1.7 +/- 0.3	1.4 +/- 0.3
PGE_2	2.5 +/- 0.2	2.5 +/- 0.3	2.2 +/- 0.2	4.6 +/- 0.9 *

* Significantly different ($P \leq 0.05$) from corresponding male value.

4.8.2 Methods

Normotensive female albino Wistar rats and female GH rats, aged 2-3 months and weighing 200-250g and 180-200g respectively were used. The mesenteric arterial bed was prepared according to McGregor (1965) with some modifications. The superior mesenteric artery was cannulated about 2 cm distal to the aorta. The entire mesentery was carefully dissected away from the gut and the vascular bed was placed in a heated chamber at 37°C, where it was perfused at 4 ml/min with oxygenated (95% O_2, 5% CO_2) McEwen's solution. The vascular bed was perfused for 30 min to allow for equilibration of the tissue after the trauma of dissection.

NA and AII (0.1 μg and 1.0 μg) were given as a bolus injection in a random order. Samples were collected at 1 min intervals for the 2 min before stimulation; for the 4 min immediately after stimulation and for a further two periods, 8 min and 12 min after stimulation. Pressor responses were recorded on a Polygraph recorder via a Statham pressure transducer. 6-Keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 were measured by RIA, without extraction. It has been shown previously that the PG content of perfusates does not differ significantly between extracted and non-extracted samples (Pipili and Poyser, 1981). The effects of NA and AII on PG release were tested for significance by comparing the 1 min period prior to stimulation to the two 1 min periods post-stimulation using Student's t-test for paired data. Significance was tested at the 5% level.

4.8.3 Results and Conclusions

6-Keto-$PGF_{1\alpha}$ was the major PG released by the mesenteric vascular bed of normotensive and GH female rats, with lower amounts of PGE_2 and $PGF_{2\alpha}$. The release of all 3 PGs declined over the duration of the experiment (60 min) in both groups of rats, as shown in Table 4.8.1.

Table 4.8.1
The release of prostaglandins (PGs) from the isolated, perfused mesenteric arterial bed of normotensive and hypertensive female rats (mean+/-s.e.m., n=4; ND- < 30 ng/min).

	Normotensive		Hypertensive	
PG ng/min	START	END	START	END
6-keto-$PGF_{1\alpha}$	2.75+/-0.51	0.58+/-0.14	2.15+/-0.38	0.78+/-0.80
PGE_2	0.71+/-0.24	0.44 (2-ND)	0.58+/-0.29	0.48+/-0.13
$PGF_{2\alpha}$	0.61+/-0.24	ND	0.63+/-0.23	0.31+/-0.08

The decline in the release of 6-keto-$PGF_{1\alpha}$ was significant ($P < 0.01$) in both normotensive and GH female rats. The amount of PGE_2 released by normotensives declined considerably, since PGE_2 reached non-detectable levels in the perfusate of 2 out of 4 animals. The amount of PGE_2 released by GH rats was not significantly lower at the end of the experiment. The amount of $PGF_{2\alpha}$

released by the mesenteric bed of normotensive females declined rapidly and reached non-detectable levels. The mesenteric bed of hypertensive females, however, showed a consistent release of $PGF_{2\alpha}$ over the duration of the experiment.

The initial amounts of PGs released per min from the mesenteric vascular bed did not differ significantly between normotensive and GH female rats (Student's t-test).

4.8.3.1 AII-stimulated PG release

AII had variable effects on the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric vascular bed of female normotensives (Figure 4.8.1) with both doses producing some stimulation if not in the first minute then in the next 3 min, although this was significant only for the 1.0 μg dose of AII. AII, at both doses, had no significant effect on the release of $PGF_{2\alpha}$ or PGE_2 (Tables 4.8.2 and 4.8.3). AII had a pressor action on the mesenteric bed of female normotensives; the 0.1 μg dose gave a 15.5 +/- 6.0% increase and the 1.0 μg dose gave a 38.0 +/- 9.7% increase in the perfusion pressure (58.3 +/- 2.2 mmHg) of the vascular bed. This was a significant increase in perfusion pressure at the higher dose of AII ($P < 0.05$).

In the mesenteric preparation from GH female rats AII again had variable effects on 6-keto-$PGF_{1\alpha}$ release and although there was some stimulation at the lower dose of

Figure 4.8.1. Angiotensin II (AII)-stimulated release of prostaglandin (PG)I_2, measured as 6-keto-$PGF_{1\alpha}$ from the isolated, perfused mesenteric arterial bed of 4 normotensive - B, and 4 New Zealand genetically hypertensive female rats - A. * compared to value immediately preceding stimulation.

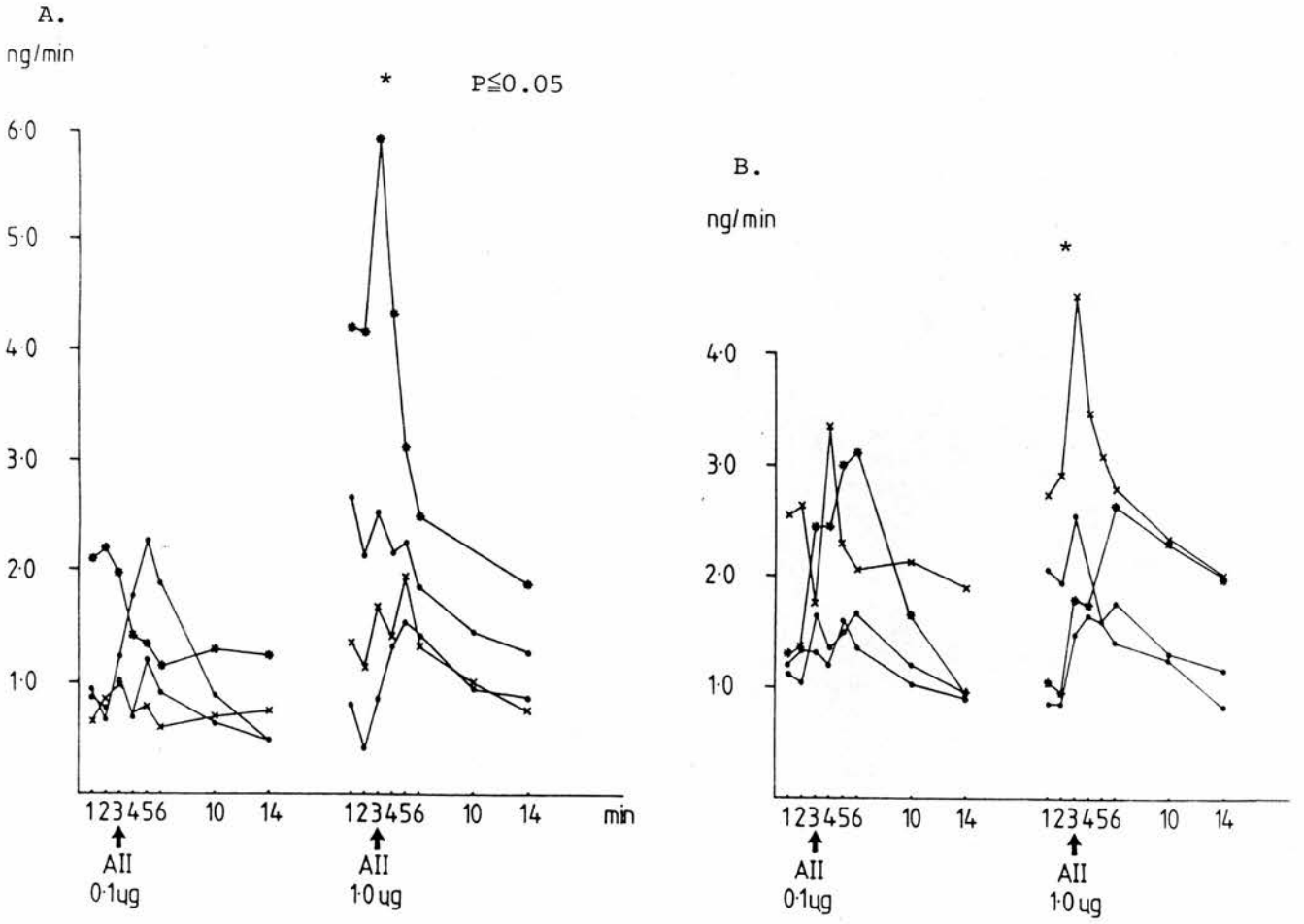


Table 4.8.2.

Angiotensin II(AII)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive female rats (ND-< 20 pg/min)

PGF2α release $ngmin^{-1}$

	0.1 µg AII ↓								1.0 µg AII ↓							
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
	ND	→							ND	→						
NORMOTENSIVE	0.75	0.75	0.70	0.30	0.32	0.42	ND	ND	1.08	0.87	1.52	0.87	0.92	0.43	0.33	0.42
FEMALES	ND	→							ND	→						
	ND	→							0.32	0.32	0.66	0.36	0.44	0.55	0.30	0.32
	0.30	0.38	0.90	0.50	0.30	0.45	0.60	0.50	0.69	0.66	1.10	0.75	0.76	0.72	0.30	0.50
HYPERTENSIVE	0.53	0.58	0.51	0.27	0.34	0.45	0.36	0.38	1.14	0.67	2.00	1.63	0.99	0.69	0.24	0.86
FEMALES	0.49	0.45	0.41	0.44	0.40	0.58	0.24	0.54	0.30	0.38	0.32	0.42	0.29	0.30	ND	ND
	0.50	0.52	0.70	0.72	0.92	0.42	0.36	0.40	0.50	0.20	0.16	0.40	0.66	0.32	0.42	0.50

Table 4.8.3.

Angiotensin II(AII)-stimulated release of prostaglandin (PG) E_2 from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive female rats (ND-< 20 pg/min)

PGE2 release $ngmin^{-1}$

	0.1 µg AII ↓								1.0 µg AII ↓							
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
	ND	→							ND	→						
NORMOTENSIVE	0.70	1.01	0.61	ND	ND	0.35	0.46	0.52	1.18	0.86	1.46	0.72	0.78	1.82	1.55	1.26
FEMALES	0.30	0.42	0.40	0.32	0.40	0.51	0.38	0.22	0.16	0.20	0.24	0.26	0.50	0.32	0.16	0.30
	ND	ND	ND	0.21	0.40	0.34	0.23	0.52	0.38	0.35	0.51	0.50	0.64	0.74	0.30	0.24
	1.10	1.46	0.16	0.35	0.18	0.32	0.35	0.32	1.16	1.43	1.65	1.24	1.14	0.99	1.36	1.50
HYPERTENSIVE	1.00	0.80	0.64	0.38	0.42	ND	ND	ND	0.32	0.66	0.46	ND	ND	ND	ND	ND
FEMALES	ND	ND	0.44	0.22	ND	ND	ND	ND	ND	ND	0.43	0.26	0.34	0.37	ND	ND
	ND	0.26	0.27	0.40	0.43	0.58	0.22	ND	ND	→						

AII, this was not significant. The 1.0 μg dose of AII produced a significant stimulation in the amount of 6-keto-$PGF_{1\alpha}$ released (Figure 4.8.1). The mesenteric bed of hypertensive females showed a similar response to normotensives with regard to PGE_2 and $PGF_{2\alpha}$ release, with neither dose of AII producing a significant stimulation in the release of these 2 PGs (Tables 4.8.2 and 4.8.3). The 0.1 μg dose of AII gave a 31.6 +/- 16.1% rise, and the 1.0 μg dose of AII gave a 61.8 +/- 25.8% rise in basal perfusion pressure (49.1 +/- 4.2 mmHg) of the mesenteric bed. Neither dose of AII produced a significant increase in perfusion pressure.

4.8.3.2 NA-stimulated PG release

Both the 0.1 μg and the 1.0 μg dose of NA produced a significant stimulation ($P < 0.05$) in the release of 6-keto-$PGF_{1\alpha}$ from the perfused mesenteric bed (Figure 4.8.2). The release of $PGF_{2\alpha}$ and PGE_2 did not change significantly after either dose of NA (Tables 4.8.4 and 4.8.5). The 0.1 μg dose of NA produced a 40.8 +/- 4.7% increase, and the 1.0 μg dose of NA a 150.4 +/- 33.2% increase in basal perfusion pressure of the mesenteric vascular bed. The increases in perfusion pressure were significant ($P < 0.05$) at both doses of NA.

A bolus injection of 0.1 μg or 1.0 μg NA produced a significant ($P < 0.05$) increase in the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed of female hypertensive rats (Figure 4.8.2). The release of $PGF_{2\alpha}$

Figure 4.8.2. Noradrenaline (NA)-stimulated release of prostaglandin (PG)I_2, measured as 6-keto-$PGF_{1\alpha}$ from the isolated, perfused mesenteric arterial bed of 4 normotensive - B, and 4 New Zealand genetically hypertensive female rats - A. * compared to value immediately preceding stimulation.

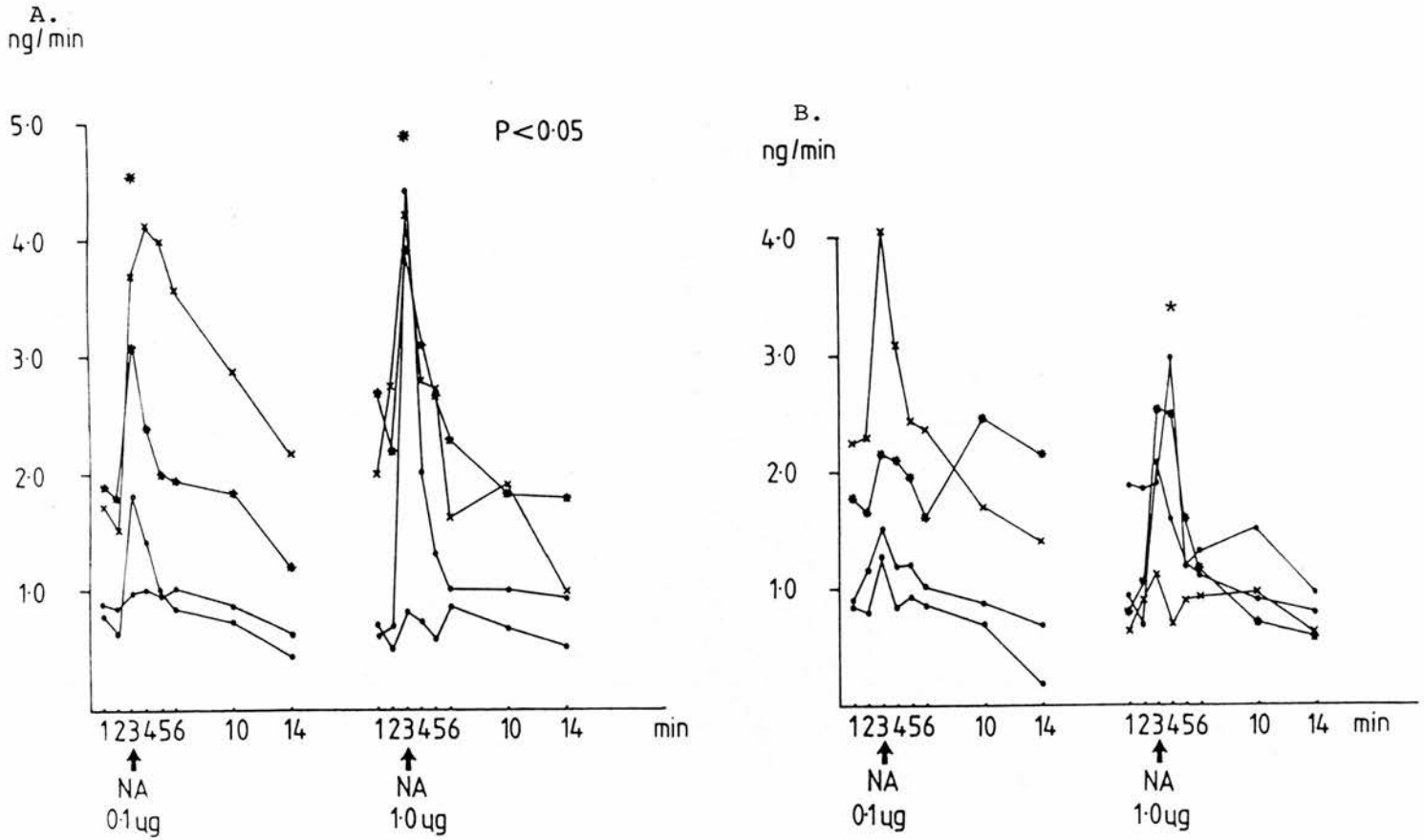


Table 4.8.4.

Noradrenaline (NA)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive female rats (ND-< 20 pg/min)

PGF2α release $ngmin^{-1}$

	0.1 µg NA ↓								1.0 µg NA ↓							
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1.	2	3	4	10	14
	ND	→							ND	→						
NORMOTENSIVE	0.64	0.53	0.44	0.40	0.70	0.68	0.35	0.75	0.19	0.86	0.85	0.33	0.29	0.30	0.18	0.43
FEMALES	ND	→							ND	0.42	0.45	0.34	0.50	0.46	0.50	0.35
	ND	→							0.16	0.62	0.22	0.22	0.27	0.28	0.22	0.64
	0.75	1.30	1.90	1.80	1.25	0.90	0.50	0.45	0.50	0.75	ND	0.57	0.69	0.45	0.50	0.21
HYPERTENSIVE	0.53	0.20	1.15	0.80	1.00	0.90	1.00	1.40	0.43	ND	0.60	0.21	0.64	0.22	0.80	0.24
FEMALES	0.82	0.46	0.43	0.28	0.64	0.42	0.49	0.45	0.18	0.24	ND	ND	0.69	0.67	0.27	0.26
	0.21	ND	0.62	0.54	0.35	0.42	0.48	0.80	0.38	0.22	1.18	0.88	0.31	0.45	0.51	0.46

Table 4.8.5.

Noradrenaline (NA)-stimulated release of prostaglandin (PG) E_2 from the isolated, perfused mesenteric arterial bed of normotensive and New Zealand genetically hypertensive female rats (ND-< 20 pg/min)

PGE2 release $ngmin^{-1}$

	0.1 µg NA ↓								1.0 µg NA ↓							
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
	ND	→							ND	→						
NORMOTENSIVE	1.28	0.79	0.22	0.65	0.44	1.71	1.25	1.15	0.38	0.22	ND	ND	0.62	ND	ND	ND
FEMALES	0.26	0.34	0.24	0.56	0.24	0.24	0.52	0.48	0.58	0.54	0.61	0.59	0.67	0.74	0.67	0.56
	0.16	0.19	0.75	0.48	ND	ND	ND	ND	ND	0.43	0.51	0.74	ND	0.21	0.35	0.39
	0.36	0.50	1.01	1.42	1.71	1.10	0.86	0.21	1.01	0.70	0.70	0.48	0.36	0.40	0.70	0.60
HYPERTENSIVE	0.42	ND	0.30	ND	0.19	ND	0.21	0.43	0.53	ND	ND	0.91	ND	ND	ND	ND
FEMALES	0.46	ND	0.63	ND	ND	ND	0.16	0.21	ND	→						
	ND	ND	0.32	0.26	ND	0.18	0.77	0.62	ND	ND	0.57	0.34	0.22	ND	ND	ND

and PGE_2 did not change significantly after either dose of NA in the mesenteric bed of female hypertensives. NA produced a rise in basal perfusion pressure (49.1 +/- 4.2 mmHg) in the mesenteric bed of hypertensives; a 52.2 +/- 26.2% increase at 0.1 μg NA and a 136.6 +/- 42.6% increase at 1.0 μg NA. This was a significant ($P < 0.05$) increase in perfusion pressure at the higher dose of NA.

In summary, AII had similar effects in both normotensive and hypertensive females with only the higher, 1.0 μg dose of AII producing a significant stimulation in the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed. The pressor effect of AII tended to be greater in the mesenteric bed of GH compared to normotensive rats, but this was not significant.

NA at both doses produced a significant increase in the output of 6-keto-$PGF_{1\alpha}$ from the perfused mesenteric bed of normotensive female rats, but only the higher (1.0 μg) dose of NA produced an increase in 6-keto-$PGF_{1\alpha}$ output in hypertensive female rats. PGE_2 and $PGF_{2\alpha}$ release were not changed markedly by the administration of NA in either group. Similarly, the vasoconstrictor response to NA did not differ between GH and normotensive females.

SECTION 4.9

A comparison of the effects of noradrenaline (NA) and angiotensin II (AII) on PG release from the perfused mesenteric arterial bed of male and female normotensive and hypertensive rats.

Table 4.9.1 compares the 6-keto-$PGF_{1\alpha}$ - releasing abilities of NA and AII in male compared to female normotensive rats.

Table 4.9.1

Comparison of the ability of noradrenaline (NA) and angiotensin II (AII) to cause a significant ($P < 0.05$) or non-significant (NS) release of 6-keto-$PGF_{1\alpha}$ from the isolated, perfused mesenteric bed of male and female normotensive rats.

	NA		AII	
	0.1 μg	1.0 μg	0.1 μg	1.0 μg
Male	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Female	$P < 0.05$	$P < 0.05$	NS	$P < 0.05$

Male and female normotensive rats were similar in their response to NA, with both doses producing a significant stimulation in the output of 6-keto-$PGF_{1\alpha}$, but not $PGF_{2\alpha}$ and PGE_2, from the mesenteric bed. There is a difference in the ability of AII to stimulate the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed, with no significant stimulation observed at the low dose of AII in females,

in contrast to the stimulation observed in male rats. Also, in contrast to females, males showed a significant stimulation in the release of PGE_2 from the mesenteric bed in response to the higher dose of AII. Neither group showed any stimulation in $PGF_{2\alpha}$ release.

Table 4.9.2 compares the 6-keto-$PGF_{1\alpha}$-releasing abilities of NA and AII in male compared to female hypertensive rats.

Table 4.9.2

Comparison of the ability of noradrenaline (NA) and angiotensin II (AII) to cause a significant ($P < 0.05$) or non-significant (NS) release of 6-keto-prostaglandin $F_{1\alpha}$ from the isolated, perfused mesenteric bed of male and female hypertensive rats.

	NA		AII	
	0.1 μg	1.0 μg	0.1 μg	1.0 μg
Male	$P < 0.05$	$P < 0.01$	NS	NS
Female	NS	$P < 0.05$	NS	$P < 0.05$

Male and female GH rats showed a similar significant increase in the release of 6-keto-$PGF_{1\alpha}$ in response to both doses of NA. Male and female hypertensive rats differed slightly in their response to AII in the mesenteric bed; no stimulation of 6-keto-$PGF_{1\alpha}$ release was observed in males at either dose of AII, but females

showed a significant stimulation in the release of 6-keto-$PGF_{1\alpha}$ at the 1.0 μg dose of AII. Male and female hypertensive rats were similar in their response to NA and AII regarding $PGF_{2\alpha}$ and PGE_2 release, with neither group of rats showing any stimulation in the release of the 2 PGs after either agonist.

SECTION 4.10

Discussion

The experiments described in this section were undertaken to determine whether there was a fundamental difference in the production of PGI_2, $PGF_{2\alpha}$ or PGE_2 in the blood vessels from normotensive compared to New Zealand genetically hypertensive rats and, within these groups, whether the production of these 3 PGs differed between male and female rats. Three parameters of PG production were investigated; total synthetic capacity in homogenates of the separated layers of aorta, basal release of PGs from the perfused aorta, and stimulated release of PGs from a resistance bed.

Unlike the majority of work on blood vessels from hypertensive rats, an increase in either the production or basal release of PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) was not observed from the vessels of GH rats. However, the most marked difference between the two groups of male rats was the increased production of $PGF_{2\alpha}$ by whole aorta, smooth muscle and endothelial cell layers; and the greater basal output of $PGF_{2\alpha}$ from the perfused aorta and mesenteric arterial bed of hypertensives compared to normotensives. The difference in the amounts of $PGF_{2\alpha}$ synthesised by aorta was not due to differences in metabolism, and is possibly related to a greater synthetic capacity of aortic endothelial and smooth muscle cells for $PGF_{2\alpha}$ production in hypertensive male

rats. Female hypertensive rats however, showed a decreased production of both 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ by the smooth muscle and endothelial cell layers but an increased production of PGE_2 compared to normotensives. In contrast to the synthetic capacity of homogenates, the basal output of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 were greater by the perfused aorta of hypertensive than normotensive females. Interestingly, the higher output of PGI_2 from the aorta of GH female rats is in agreement with previous studies.

Since the discovery of PGI_2 and its being 'labelled' as the major and most important product of AA metabolism in blood vessels, $PGF_{2\alpha}$ and PGE_2 have either been ignored or deemed unimportant. Yet, both of these PGs have potent effects on blood pressure. In the rat and dog $PGF_{2\alpha}$ is pressor; intra-arterial administration of $PGF_{2\alpha}$ increases vascular resistance, reportedly through a selective venoconstrictor action (DuCharme and Weeks, 1967; DuCharme et al., 1968). In the micro-circulation of the cat (pial vessels), topical administration of $PGF_{2\alpha}$ produces significant arterial constriction (Welch et al., 1974), and in the rat, arteriolar constrictor responses to $PGF_{2\alpha}$ have been observed in the mesocaecum (Vignera et al., 1969) and cremaster muscle (Ellis and Hutchins, 1974).

The potent vasodepressor actions of PGE_2, in all species studied, are well documented but the effects of PGE_2 are not universally vasodepressor in different vascular beds

of the rat. In the splenic vasculature and cremaster muscle, PGE_2 produces vasodilation (Malik, 1979; Messina et al., 1974) but the situation in the kidney is less clear. Inhibition of PG synthesis produced vasoconstriction, suggesting that an influence of vasodilator PGs had been reduced (Dusing et al., 1977), but direct administration of PGE_2 into the rat kidney increased vascular tone (Malik and McGiff, 1975; Gerber and Neiss, 1979).

The output of $PGF_{2\alpha}$ from the kidney is higher than normal in humans with essential hypertension (Weber et al., 1979), and in the SHR an increased renal $PGF_{2\alpha}$ formation was found even before overt hypertension had developed (Ahnfelt-Ronne and Arrigoni-Martelli, 1978). PGE_2 output however, did not change. These observations made using the kidney from hypertensive rats and the findings made using the aorta in the present study, support the idea that an alteration in the pathway of PG formation from PGE_2 to $PGF_{2\alpha}$ may lead to an increase in blood pressure, and that it is the ratio of these PGs that is critical for blood pressure control rather than the absolute amounts of vasodilator PGs.

As discussed in the introduction to this section the release of PGs may be important in the attenuation of the vasoconstrictor responses to certain hormones. PGE_2 is believed to act by reducing the amount of transmitter released, whereas PGI_2 does not act in this way and probably has a direct action on myogenic tone. The

response of PG output to NA was similar in normotensive and hypertensive male rats i.e. an increase in the release of 6-keto-$PGF_{1\alpha}$, and a tendency for increased PGE_2 release, but the pressor response to NA was greater in GH than normotensive male rats implying that PGI_2 can not be the only factor involved in the control of the vasoconstrictor response to NA.

PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) and PGE_2 are released from the mesenteric bed of normotensive males in response to AII, in agreement with other authors (Desjardins-Giasson, Gutkowa, Garcia and Genest, 1982; Dusting, Mullins and Nolan, 1981), but are not released from the same preparation of hypertensive males. Interestingly, hypertensives showed a greater pressor response to AII than normotensives suggesting that there may be a deficiency in these proposed aspects of the control of vascular tone by PGE_2 and/or PGI_2 in the GH male rat.

However, the results from female rats do not agree with this hypothesis since AII (1.0 μg) stimulated 6-keto-$PGF_{1\alpha}$ output in female normotensive and hypertensive rats, unless there is a fundamental sex difference in the control of vascular tone. Certainly, the pressor response to AII (1.0 μg) did not differ significantly between normotensive and hypertensive, female rats. Comparing males to females, the pressor effects of NA and AII were always smaller in females (normotensive or hypertensive) but this was not significa nt.

It has been known for a long time that vascular hyperresponsiveness to vasoconstrictor agents is a characteristic of human essential hypertension, and of other forms of experimental hypertension in rats (Somlyo & Somlyo, 1970), However, despite extensive investigation, the relationship between elevated blood pressure and hyperresponsiveness has not been defined. A recent study has shown that young GH rats show hyperresponsiveness before the blood pressure is significantly elevated. Delaying the development of hypertension by bilateral renal denervation does not lessen the hyperresponsiveness, but 1 kidney, 1 clip renal hypertensive rats show a much smaller increase in vascular hyperresponsiveness than age-matched controls (Baer, 1984). These results indicate that vascular hyperresponsiveness of GH rats is a primary characteristic and is not due solely to an elevated blood pressure.

The observation that basal $PGF_{2\alpha}$ release was greater from the mesenteric bed of hypertensive male and female rats compared to their normotensive controls is of considerable interest in relation to the potent vasoconstrictor actions of $PGF_{2\alpha}$. In the dog, $PGF_{2\alpha}$ is a potent mesenteric vasoconstrictor (Chapnick et al., 1978). It also enhances the vasoconstrictor response following nerve stimulation (Hedquist, 1976) and there are reports that AII induces the release of $PGF_{2\alpha}$ from

the canine kidney (Dunn et al., 1978). Thus, although there was no actual stimulation of $PGF_{2\alpha}$ release in the mesenteric bed of hypertensive rats after NA and AII, the observation that $PGF_{2\alpha}$ was always present in higher amounts in these animals may be of relevance to the greater pressor response found in these animals. Whether the increased capacity for $PGF_{2\alpha}$ synthesis and the increased basal output of $PGF_{2\alpha}$ from perfused vessels is connected with the increased blood pressure found in these animals requires further investigation.

Blood pressure increases with increasing age in rats and humans. The experiments described in the next section were designed to investigate the profile of PG production and basal output from blood vessels of male and female aged rats, established to have significantly higher blood pressures than young male and female rats.

SECTION 5.0

Measurement of the PG synthetic capacity of homogenates of aorta, the basal release of PGs from the perfused aorta and the stimulated release of PGs from the perfused mesenteric arterial bed of male and female, young and old rats.

5.1.1 Introduction

A complex interrelationship exists between blood pressure and arterial disease, hypertension increasing the susceptibility of blood vessels to arteriosclerosis. It is generally recognised that blood pressure increases with age and that the incidence of coronary heart disease and thrombosis also increases with age in both men and women. The mortality and morbidity from cardiovascular diseases is much greater in males than in pre-menopausal females (Kuller, 1976). Studies to date do not show unequivocally that these increases in blood pressure and thrombotic tendency with increasing age are related to PG mediated effects. It has been shown that 15-hydroperoxyarachidonic acid (15-HPAA), a lipid peroxide, is a potent and selective inhibitor of PGI_2 generation by porcine vessel wall microsomes or by fresh vascular tissue (Moncada et al., 1976). Other fatty acid peroxides and their methyl esters show similar effects (Salmon, Smith, Flower, Moncada and Vane, 1978). Lipid peroxidation induced by free radical formation is known

to occur in the ageing process (Slater, 1972) and it is reasonable to suggest that PGI_2 generation could be reduced in aged blood vessels because of the effects of greater concentrations of lipid peroxides.

Pace-Asciak and Carrara (1979) and Panganamala et al., (1981) have shown that PGI_2 formation by aortic homogenates or intact aorta increases with age in the rat. However, these authors were studying a very limited age range using rats of up to 20 weeks of age and were not looking at the situation in the old animal. Aortic smooth muscle cells from old rats produced less PGI_2 in culture than those obtained from young rats and this was due to a specific decrease in PGI_2 synthetase activity. These cells from old rats were found to produce more PGE_2 than PGI_2 (Chang et al., 1980). Using bovine smooth muscle and endothelial cells *in vitro* (Ager et al., 1982) it was observed that during sub-culture the ability to generate PGI_2 decreased while PGE_2 formation increased. It is not known if these changes are due to specific effects of lipid peroxides on PGI_2 synthetase. The experiments described in this section were carried out to determine the profile of PG production and release in aorta and mesenteric bed of old (12-14 months) male and female rats.

5.1.2 Measurement of the PG synthetic capacity of separated layers of the aorta from young and old male rats.

Introduction

In this and in following sub-sections, the appropriate data for young rats is taken from Section 4.

5.1.3 Methods

Male albino Wistar rats aged 2-3 months weighing 200-250g, and male albino Wistar rats aged 12-14 months and weighing 680-730g were used. The animals were housed under controlled conditions and were allowed a standard diet and water. The aged rats (12-14 months old) were bought from the supplier at the age of 2 months and were kept in the departmental animal house until they reached the age of 12-14 months.

5.1.4 Blood Pressure measurement

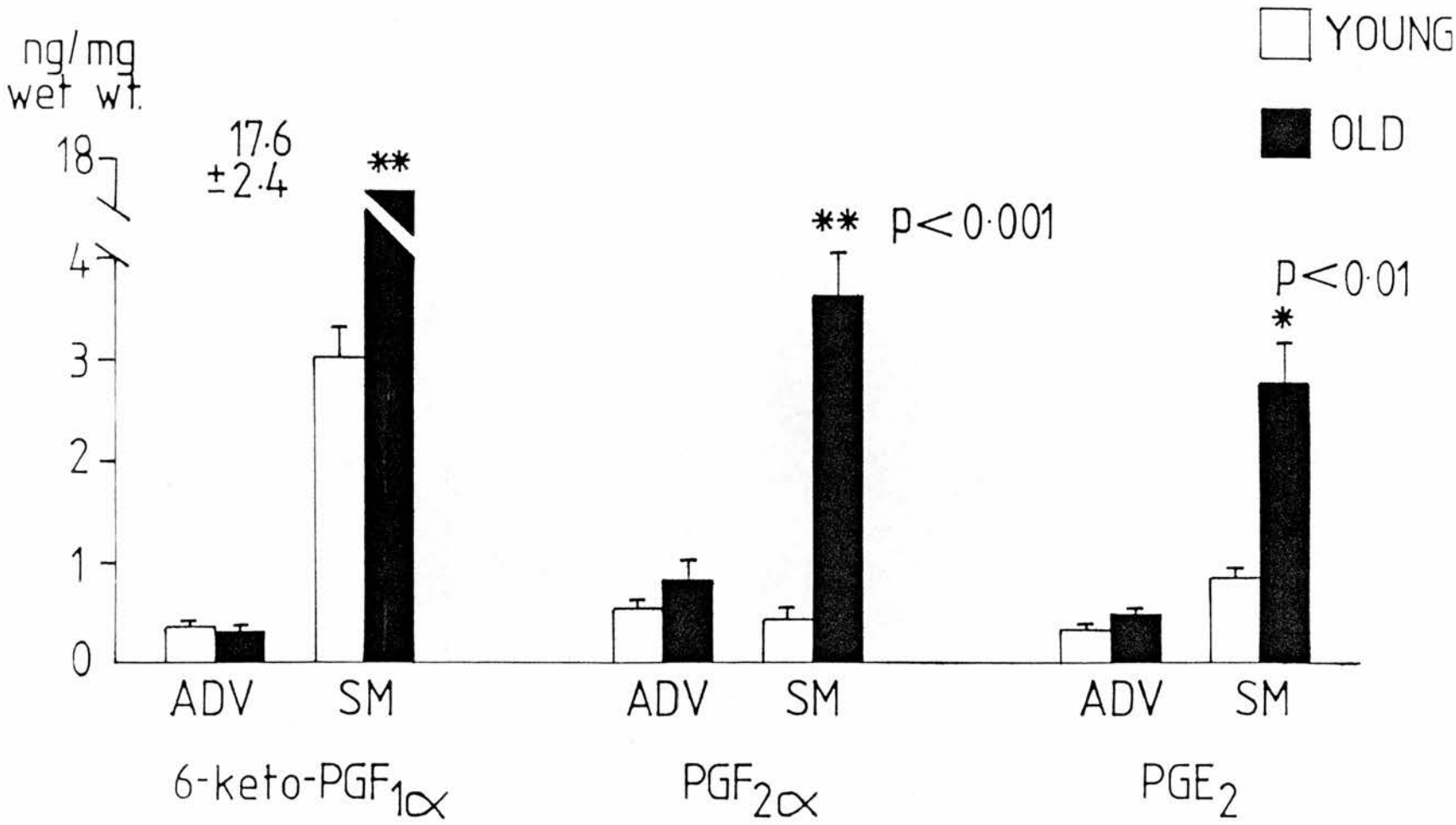
Measurements were made in the same cage order and at the same time of day (11:00h to 1300:h), every day for 2 weeks. Systolic blood pressure was measured indirectly using the tail cuff method as described in detail in Section 4.1.3. The restraining holder for the aged rats was adapted to accomodate their rather large size. After consistent blood pressure measurements had been obtained, the animals were killed (6 in each group) and a 2.5 cm length of the thoracic aorta was removed. The aorta was

rinsed in Krebs solution to remove any blood, was opened longitudinally and with its endothelial surface uppermost, was pinned out on a plastic petri-dish. The endothelial cells were carefully scraped off with a scalpel blade and were suspended in 5ml Krebs solution. The adventitia and smooth muscle layers were also separated with a scalpel blade and then weighed. The adventitia and smooth muscle layers were homogenised separately in 2.5ml Krebs solution. The homogeniser was washed with 2.5ml Krebs solution and the homogenate and washings were added to a 25 ml conical flask. The endothelial cell layer was left as a cell suspension in 5ml Krebs solution. Arachidonic acid (2 μg/ml) was added to each flask and the flasks were incubated for 60 min. PGs were extracted with ethyl acetate as described in Section 2.4. Samples in ethyl acetate were stored at -20° C until assayed for PG content by RIA as detailed in Section 2.5. The results from young and old rats were compared using Student's t-test and significance was tested at the 5% level.

5.1.5 Results

The mean (+ s.e.m., n=6) systolic blood pressure of the young male rats was 104.8 + 4.3 mmHg and that of the old male rats was 179.7 + 9.2 mmHg. This was a significant increase ($P < 0.001$) in the blood pressure of the old male compared to the young male rats. As can be seen from Figure 5.1.1, homogenates of adventitia from old and young males produced similar amounts of the 3 PGs. There

Figure 5.1.1. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by homogenates of adventitia (ADV) and smooth muscle (SM) from young (2-3 months) and old (12-14 months) male rats.

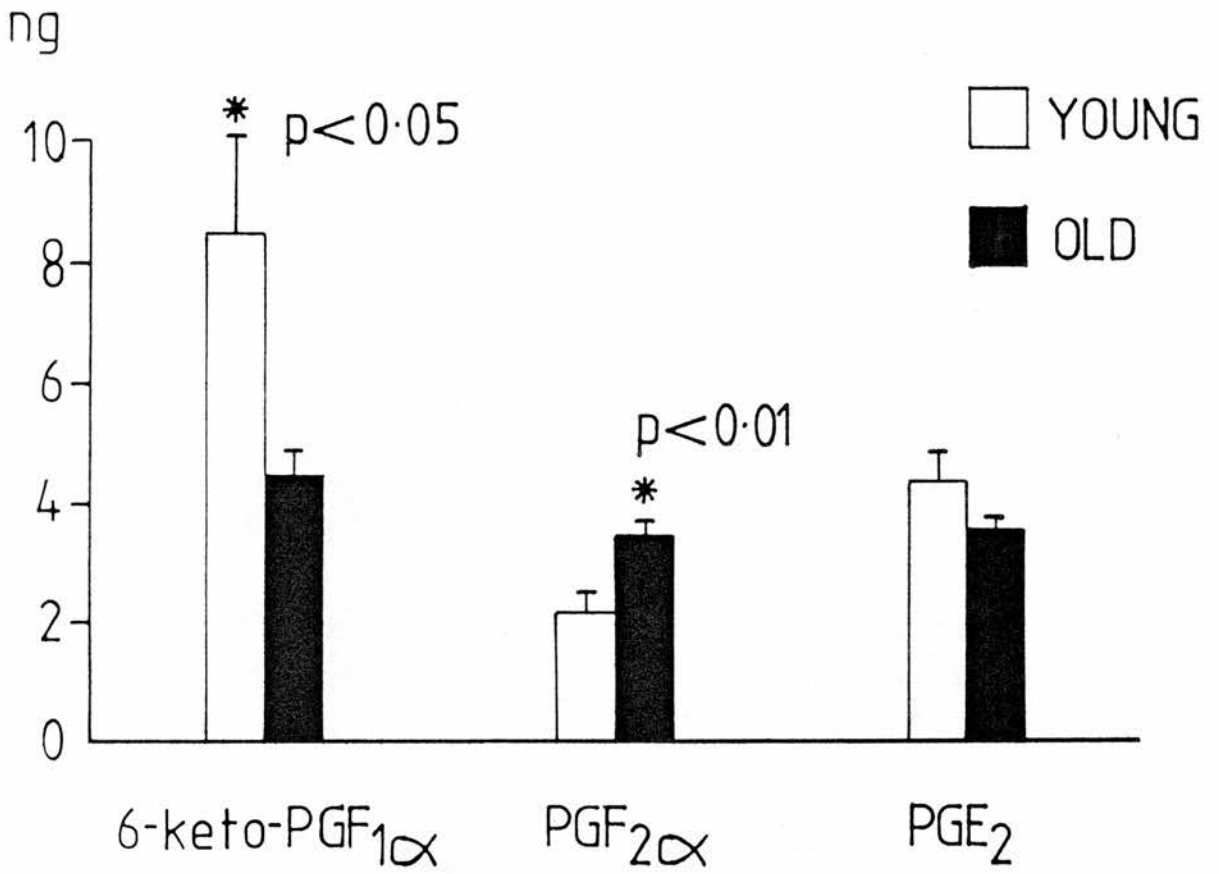


was no significant difference in PG production by adventitia of old compared to young male rats. The capacity of the smooth muscle layer for PG synthesis was much greater than for the adventitia in both young and old rats. 6-Keto-$PGF_{1\alpha}$ was the major PG produced by the smooth muscle layer of young male rats with lesser amounts of PGE_2 and $PGF_{2\alpha}$. 6-Keto-$PGF_{1\alpha}$ was also the major PG produced by the smooth muscle layer of old rats with considerably lesser amounts of PGE_2 and $PGF_{2\alpha}$. There was a marked increase in the production of 6-keto-$PGF_{1\alpha}$ ($P < 0.001$), $PGF_{2\alpha}$ ($P < 0.001$) and PGE_2 ($P < 0.01$) by homogenates of smooth muscle layer of old compared to young male rats. The endothelial cell layer from young rats produced mainly 6-keto-$PGF_{1\alpha}$ with lower amounts of PGE_2 and $PGF_{2\alpha}$ (Fig. 5.1.2). The endothelium from old rats however, produced similar amounts of the 3 PGs. There was a significant ($P < 0.05$) decrease in the production of 6-keto-$PGF_{1\alpha}$ and a significant increase ($P < 0.01$) in the production of $PGF_{2\alpha}$ by the endothelium of old compared to young male rats. PGE_2 production did not differ significantly between the 2 groups.

5.1.6 Conclusions

Consistent measurements of systolic blood pressure were obtained from old male rats and it was established that the blood pressures of these old rats were significantly greater than that of young male rats. The old rats therefore provided an additional means of studying the effects of a naturally elevated blood pressure on vascular PG production.

Figure 5.1.2. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by an endothelial cell suspension from a 2.5cm length of aorta from young (2-3 months) and old (12-14 months) male rats.



The most striking difference in PG production by the aorta of old compared to young male rats was the marked increase in the production of all 3 PGs by homogenates of smooth muscle from old male rats. The increase in 6-keto-$PGF_{1\alpha}$ was 6-fold, the increase in PGE_2 was 3-fold; but the greatest increase was in $PGF_{2\alpha}$ production which was 8-fold. The profile of PG production by the endothelium of old rats was also considerably different to that of young male rats. There was a change in the 6-keto-$PGF_{1\alpha}$: $PGF_{2\alpha}$ ratio from 3.9 : 1.0 in the endothelium of young rats to 1.3 : 1.0 in the endothelium of old male rats. PGE_2 production was not different between the two groups. Thus, the profile of PG production by smooth muscle and endothelium from old rats was changed in favour of $PGF_{2\alpha}$. These findings are not in agreement with Chang et al., 1980, using cultured smooth muscle cells, who found that smooth muscle cells from 24 month old rats produced less PGI_2 in culture than those from 12 month old rats. However, a later study by the same authors, Chang et al., 1983, showed that PGI_2 release from aortic rings of mature (12 month) rats is increased compared to young (2 month) rats, but is significantly decreased in senescent (24 month) rats compared to young rats. So the rats used in the present study are comparable to the mature rats used in the study of Chang et al., 1983 and show a similar trend in producing greater amounts of 6-keto-$PGF_{1\alpha}$ from the aorta than young male rats. It is not possible to comment on the increased production of $PGF_{2\alpha}$ by the aorta of old male rats in the present study as this PG was not measured by the previous authors.

SECTION 5.2

Measurement of unstimulated PG release from the isolated perfused aorta of young and old male rats.

5.2.1 Introduction

As discussed in Section 4.4.4, the perfusion of an intact vessel with collection of the perfusate and subsequent measurement of the PGs present probably gives a good indication of PG release *in vivo*. The experiments described in this sub-section were carried out to determine the profile of PG release from the aorta of young and old male rats.

5.2.2 Methods

Male albino Wistar rats aged 2-3 and 12-14 months, and weighing 250-300g and 680-730g respectively, were used. The animals were housed as previously. Six animals from each group were killed and the thoracic aorta was cannulated. A 2.5 cm length of aorta was carefully dissected free of surrounding tissue and was removed to a heated (37°C) chamber and was perfused with oxygenated Krebs solution at a rate of 5ml/min. After the preparation had equilibrated for 30 min the perfusate was collected for 30 min and was extracted in the same way as described for homogenates in Section 2.4. Samples were

stored in ethyl acetate until assayed for 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 content as detailed in Section 2.5. The results were compared using Student's t-test for unpaired data and significance was tested at the 5% level.

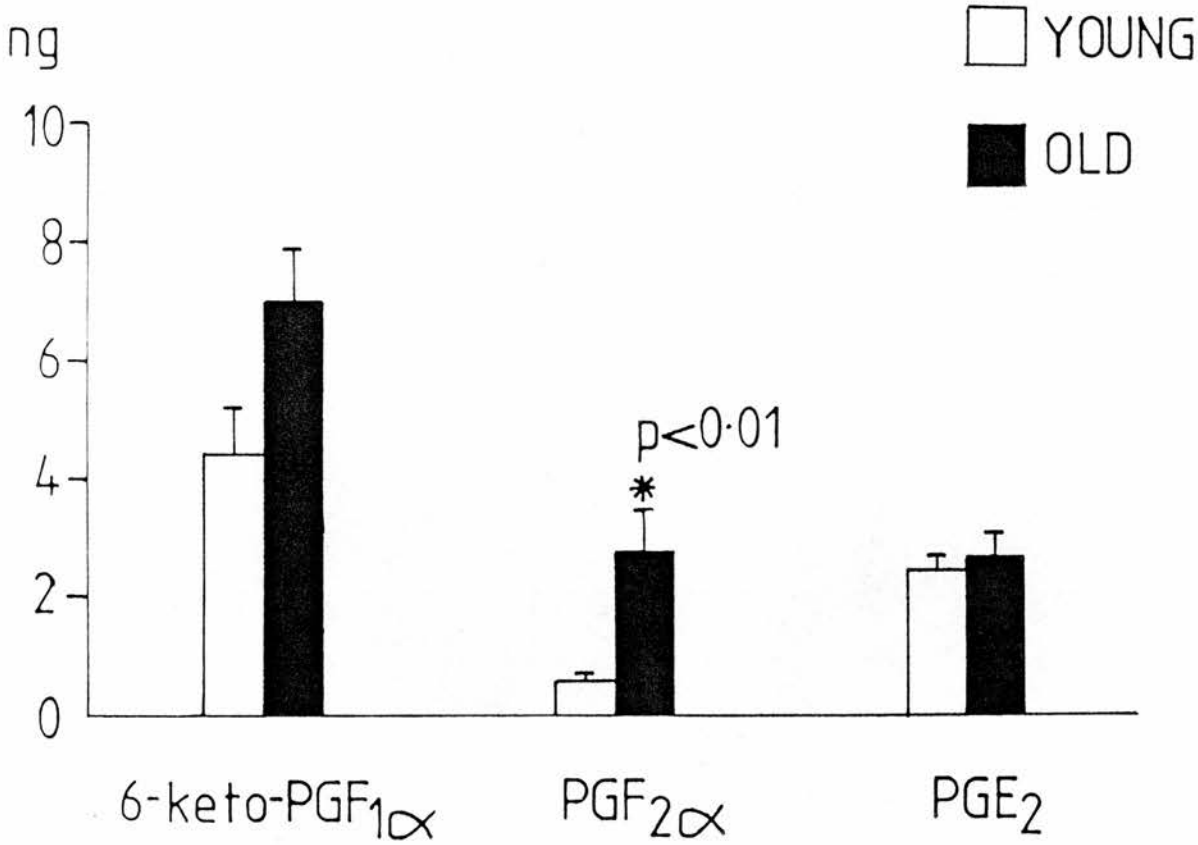
5.2.3 Results

Figure 5.2.1 compares the basal release of PGs during a 30 min period from the aortae of young and old male rats. 6-Keto-$PGF_{1\alpha}$ was the major PG released by the isolated, perfused aorta of young rats followed by lower amounts of PGE_2 and considerably lower amounts of $PGF_{2\alpha}$. The perfused aorta from old rats also released mainly 6-keto-$PGF_{1\alpha}$ and lesser, but almost equal amounts of $PGF_{2\alpha}$ and PGE_2. There was a significantly greater ($P < 0.01$) quantity of $PGF_{2\alpha}$ released from the aorta of old compared to young male rats. 6-Keto- $PGF_{1\alpha}$ release tended to be greater from the aorta of old rats but this was not significant. PGE_2 release was not significantly different between the two groups.

5.2.4 Conclusions

The perfused aorta of old male rats showed a different profile of PG release compared to young rats with a considerable increase in the release of $PGF_{2\alpha}$ being observed in the old male rats. This resulted in a change in the 6-keto-$PGF_{1\alpha}$: $PGF_{2\alpha}$ ratio. In young rats this ratio was 8.5 : 1.0 and in old rats it was 2.6 : 1.0. This change in the 6-keto-$PGF_{1\alpha}$: $PGF_{2\alpha}$ ratio was

Figure 5.2.1. Mean (+/-s.e.m.,n=6) amounts of prostaglandins released from a 2.5cm length of isolated, perfused aorta from young (2-3 months) and old (12-14 months) male rats.



possibly a reflection of the increased synthetic capacity of the smooth muscle homogenates and the endothelial cell layers from old males to produce $PGF_{2\alpha}$.

SECTION 5.3.

Measurement of stimulated PG release from the isolated, perfused mesenteric arterial bed of young and old male rats.

5.3.1 Introduction

There does not appear to be any information in the literature on the effects of pressor hormones on the release of PGs from any vascular bed from aged rats. As genetically hypertensive male rats show an increased pressor response to both NA and AII and appear to have a deficiency in the release of 6-keto-$PGF_{1\alpha}$ in response to AII compared to normotensive males (see Section 4.5.3.1), it seemed of interest to establish the PG-releasing abilities of NA and AII in old male rats which were found to have systolic blood pressures in excess of those of young normotensive and also young hypertensive male rats.

5.3.2 Methods

Young albino Wistar male rats aged 2-3 months, weighing 200-250g and similar old male rats aged 12-14 months, weighing 680-730g were used. The mesenteric arterial bed was prepared according to McGregor, (1965) with some

modifications and this is described in detail in Section 4.5.2. The vascular bed was perfused at 5ml/min with oxygenated (95% O_2, 5% CO_2) McEwen's solution. After an equilibration period of 30 min, NA and AII (0.1 μg and 1.0 μg) were given as a bolus injection in a random order. Samples were collected at 1 min intervals for the 2 min before stimulation, for the 4 min immediately after stimulation and for a further two periods, 8 min and 12 min after stimulation. Pressor responses were recorded on a Polygraph recorder via a Statham pressure transducer. 6-Keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 content of the perfusion fluid was measured by RIA, without extraction (Section 2.5). The effects of NA and AII on PG release were tested for significance by comparing the 1 min period prior to stimulation with the two 1 min periods post-stimulation using Student's t-test for paired data. Significance was tested at the 5% level.

5.3.3 Results

6-Keto-$PGF_{1\alpha}$ was the major PG released by the mesenteric bed of young male rats with considerably less $PGF_{2\alpha}$ and PGE_2 being released. The amounts of $PGF_{2\alpha}$ released into the perfusate during the 1 min collection period were below the detection limit of the assay (< 0.30 ng/ml). 6-Keto-$PGF_{1\alpha}$ was also the major PG released from the mesenteric bed of old male rats followed by lower amounts of $PGF_{2\alpha}$ and PGE_2. In contrast to the situation in young rats, there was sufficient $PGF_{2\alpha}$ in the perfusate from old rats to be measured. The amounts of 6-keto-$PGF_{1\alpha}$ and

PGE_2 were greater ($P < 0.05$) at the start of the experiment in the perfusates from old male compared to young male rats. The amounts of all 3 PGs released declined over the duration of the experiment (60 min) in both groups of rats and this was significant ($P < 0.05$) for 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ in old, but not young male rats.

5.3.3.1 AII-stimulated PG release

Both the low (0.1 μg) and high (1.0 μg) doses of AII caused an increase in the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed of young male rats and this was significant ($P < 0.05$) at 1.0 μg AII (Fig. 5.3.1). Neither dose of AII produced any stimulation of $PGF_{2\alpha}$ release above non-detectable levels (Table 5.3.2). There was some stimulation of PGE_2 release at the 0.1 μg dose of AII but this was not significant (Table 5.3.3). The higher dose of 1.0 μg AII, however, produced a significant increase ($P < 0.01$) in the release of PGE_2 from the mesenteric bed of young male rats. AII had a pressor effect on the vascular bed producing a 28.8+/-9.8% increase in the basal perfusion pressure (42.5+/-3.7 mmHg) at 0.1 μg AII and an increase of 57.2+/-7.7% at the 1.0 μg dose of AII. This was a significant increase ($P < 0.01$) in perfusion pressure at the 1.0 μg but not the 0.1 μg dose of AII.

In the mesenteric vascular bed from old males both the

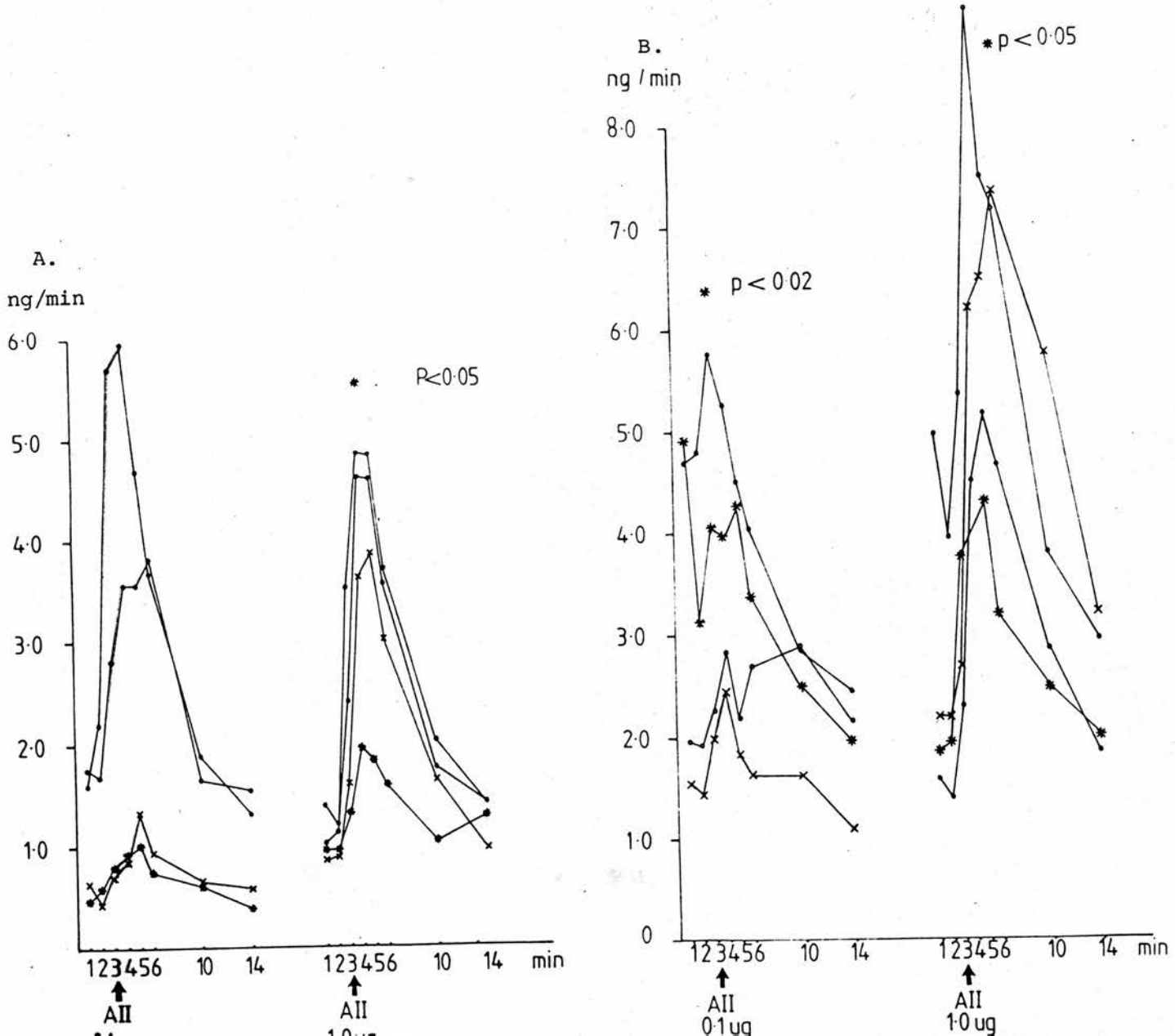

A.
ng/min
6·0
5·0
4·0
3·0
2·0
1·0
P<0·05
1 2 3 4 5 6 10 14 min
AII
AII
B.
ng / min
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
p < 0·02
p < 0·05
1 2 3 4 5 6 10 14 min
AII
0·1 ug
AII
1·0 ug

Table 5.3.2

Angiotensin II(AII)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated, perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) male rats (ND < 30 pg/min)

	$PGF_{2\alpha}$ release ng/min															
			0.1 µg AII ↓								1.0 µg AII ↓					
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
YOUNG MALES	ND →								ND →							
	ND →								ND →							
	ND →								ND →							
	ND →								ND →							
	ND →								ND	ND	ND	0.66	0.42	0.39	0.36	ND
OLD MALES	1.77	1.66	1.91	1.78	1.26	1.07	0.88	0.49	ND	0.32	0.46	0.49	0.80	0.61	0.56	0.24
	0.91	0.78	0.44	0.29	0.24	0.44	0.41	0.58	0.66	0.44	0.89	0.57	0.25	0.28	ND	ND
	0.42	0.36	0.60	0.42	0.30	0.63	0.68	0.67	1.73	1.52	1.85	1.66	1.46	1.25	0.80	1.02

Table 5.3.3.

Angiotensin II(AII)-stimulated release of prostaglandin (PG) E_2 from the isolated perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) male rats (ND < 30 pg/min)

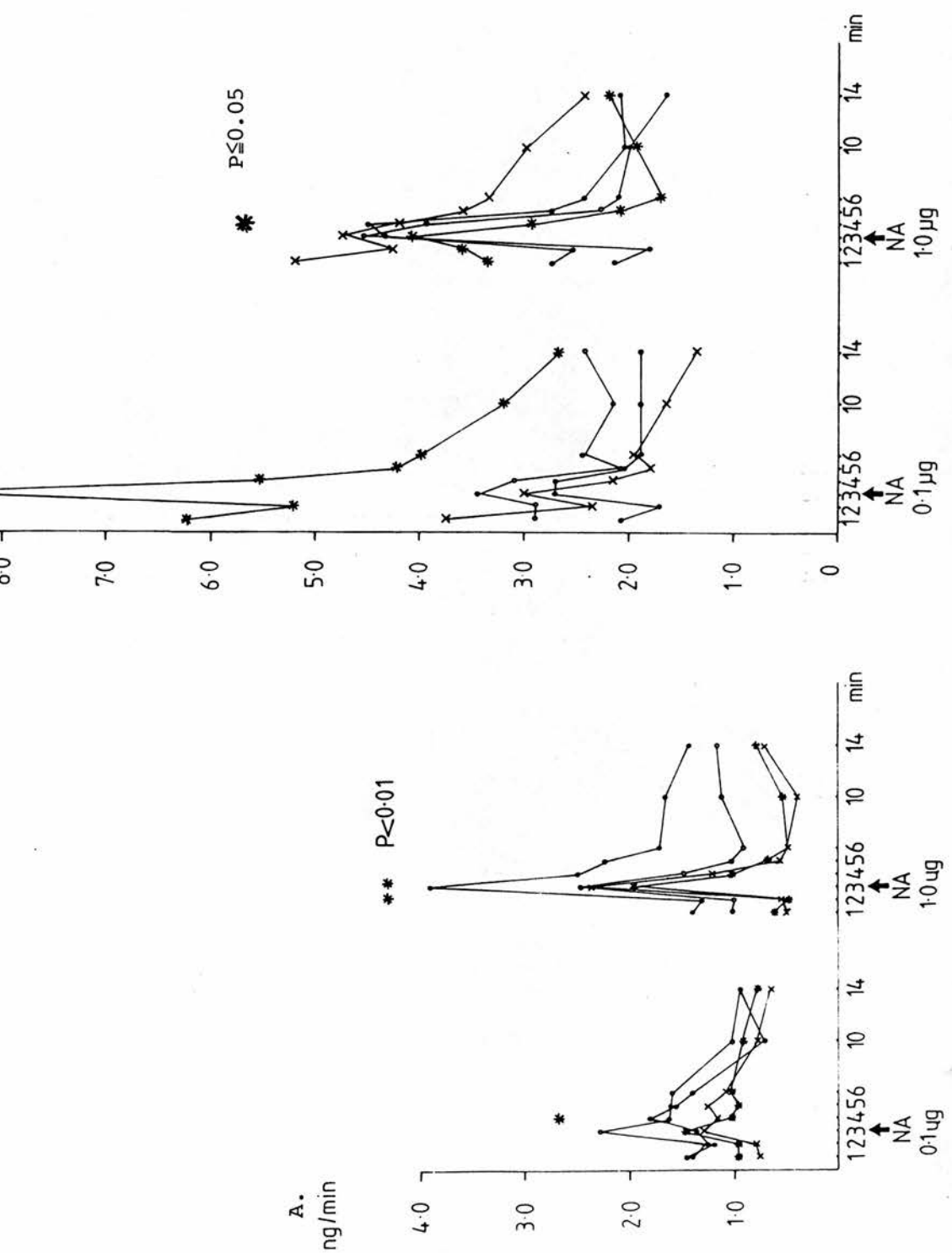
	PGE_2 release ng/min															
			0.1 µg AII ↓								1.0 µg AII ↓					
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
YOUNG MALES	0.50	0.59	0.22	0.22	0.46	0.37	0.37	ND	0.26	0.38	1.20	1.02	0.77	0.82	0.69	ND
	0.34	0.43	0.70	ND	ND	0.43	0.85	0.60	ND	0.40	0.77	0.77	0.60	0.69	0.37	0.40
	0.67	0.67	1.26	0.29	0.60	0.54	0.77	0.64	0.52	0.58	1.32	1.33	0.64	0.72	0.70	0.78
	0.80	0.82	0.74	1.33	0.94	0.80	ND	ND	0.81	0.66	0.46	1.08	0.98	0.50	0.90	1.06
OLD MALES	0.40	0.35	0.34	0.40	0.46	0.52	0.31	0.32	0.48	0.33	0.45	0.30	0.53	0.42	0.40	0.51
	0.92	0.86	1.00	0.85	0.56	0.79	0.52	0.71	0.44	0.26	0.45	0.40	0.61	0.39	0.50	0.58
	0.90	1.03	1.12	0.82	0.94	1.11	0.90	0.63	0.66	0.44	0.68	0.57	0.25	0.28	ND	ND
	0.98	1.10	1.58	1.24	0.86	1.61	2.10	1.65	2.54	1.74	2.44	2.37	1.95	1.70	1.58	1.72

doses of AII produced a significant increase ($P < 0.05$) in the basal release of 6-keto-$PGF_{1\alpha}$. There was no significant change in the release of $PGF_{2\alpha}$ and PGE_2 at either dose of AII from the mesenteric bed of old males (Tables 5.3.2 and 5.3.3). AII had a pressor effect on the vascular bed of old rats with the 0.1 μg dose causing a 24.1+/-16.8% increase and the 1.0 μg dose giving a 83.0+/-21.3% increase in basal perfusion pressure. The increase in perfusion pressure was significant ($P < 0.05$) at the 1.0 μg dose of AII. There was no significant difference in the magnitude of the pressor responses to AII in young male compared to old male rats at either dose.

5.3.3.2 NA-stimulated PG release

Both doses of NA produced a significant increase (0.1 μg NA; $P < 0.05$, 1.0 μg NA; $P < 0.01$) in the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed of young male rats (Figure 5.3.2). NA did not produce any increase in the amount of $PGF_{2\alpha}$ released to detectable levels (Table 5.3.4). Similarly, NA at either dose, had no effect on the release of PGE_2 from the mesenteric bed of young male rats (Table 5.3.5). Noradrenaline, at 0.1 μg produced a 63.2+/-34.7% increase, and 1.0 μg a 174+/-4.4% increase in perfusion pressure. The increase in perfusion pressure was significant ($P < 0.001$) at the 1.0 μg dose of NA.

In the mesenteric vascular bed of old male rats, both doses of NA produced a significant ($P < 0.05$) stimulation


B.
ng/min
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
P≦0.05
123456
NA
0·1µg
1·0µg
10
14
min
A.
ng/min
4·0
3·0
2·0
1·0
**
P<0·01
*
123456
NA
0·1µg
1·0µg
10
14
min

of 6-keto-$PGF_{1\alpha}$ release . There was no significant stimulation of $PGF_{2\alpha}$ or PGE_2 release after either dose of NA (Tables 5.3.4 and 5.3.5). The 0.1 μg dose of NA produced a 211.7+/-41.0% increase, and the 1.0 μg dose a 367.7+/-64.6% increase in basal perfusion pressure. These increases in perfusion pressure were significant at the 0.1 μg ($P < 0.05$) and at the 1.0 μg ($P < 0.01$) dose of NA. The increases in perfusion pressure induced by both doses of NA were significantly greater ($P < 0.05$) in old compared to young male rats.

5.3.4 Conclusions

AII and NA caused a similar response in the mesenteric vascular bed of both old and young male rats, producing a significant increase in the release of 6-keto-$PGF_{1\alpha}$. However, AII (1.0 μg) produced a significant increase in the release of PGE_2 from the mesenteric bed of young, but not old male rats. The pressor response to AII was similar in young and old male rats, but both doses of NA produced a considerably greater pressor response in the mesenteric bed of old compared to young male rats. The increased pressor response to NA in old rats does not appear to be due to a deficiency in the release of PGI_2.

Table 5.3.4.

Noradrenaline (NA)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) male rats (ND < 30 pg/min)

$PGF_{2\alpha}$ release ng/min

			0.1 µg NA ↓								1.0 µg NA ↓							
TIME : MIN	-1	-2	1	2	3	4	10	14		-1	-2	1	2	3	4	10	14
YOUNG MALES	ND →									ND →							
	ND →									ND →							
	ND →									ND →							
	ND →									ND →							
OLD MALES	ND →									1.03	0.91	0.99	0.76	0.67	0.37	ND	ND
	0.54	0.50	0.52	0.40	0.62	0.24	0.56	0.64		0.93	0.93	1.14	1.26	0.57	0.42	0.52	0.54
	1.63	1.38	2.00	1.16	1.13	0.78	0.89	0.47		0.74	0.47	0.42	0.46	0.45	0.42	0.42	0.63
	0.99	0.82	0.98	0.96	0.60	0.62	0.61	0.79		0.64	0.50	0.48	1.23	0.70	0.43	0.48	0.45

Table 5.3.5.

Noradrenaline (NA)-stimulated release of prostaglandin (PG) E_2 from the isolated perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) male rats (ND < 30 pg/min)

PGE_2 release ng/min

			0.1 µg NA ↓								1.0 µg NA ↓						
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14	
YOUNG MALES	ND	ND	ND	0.61	0.52	ND	ND	ND	ND	ND	0.54	0.46	0.60	0.64	0.24	0.30	
	ND	ND	ND	0.90	0.67	0.41	0.90	0.82	0.64	0.30	ND	0.86	0.55	0.46	0.66	ND	
	0.52	ND	1.17	0.44	0.50	0.78	0.41	ND	0.60	0.94	0.89	1.06	0.24	0.22	0.19	0.37	
	0.96	ND	ND	0.78	0.53	0.72	0.37	ND	0.34	0.53	0.24	0.51	0.62	ND	ND	ND	
OLD MALES	0.20	0.36	0.46	0.24	0.32	0.40	0.35	0.50	0.81	0.68	0.97	0.69	0.65	0.62	0.57	0.32	
	0.37	0.45	0.58	0.59	0.76	0.46	0.34	0.47	0.52	0.54	0.89	1.36	0.41	0.41	0.28	0.52	
	1.64	1.41	2.38	1.84	1.53	1.21	1.02	0.90	0.74	0.47	0.56	0.46	0.45	0.42	0.42	0.34	
	1.47	1.50	1.73	1.57	1.46	1.36	1.80	1.84	1.99	1.25	2.96	2.50	1.45	1.47	1.68	1.14	

SECTION 5.4

Measurement of PG production in the separate layers of aorta from young (2-3 months) and old (12-14 months) female rats.

5.4.1 Introduction

The experiments described in Sections 2 and 3 have shown that there are appreciable differences in the production of PGs by the vascular tissues of male and female rats. The production of 6-keto-$PGF_{1\alpha}$ is greater by homogenates of aorta from males compared to females. In the separated layers of aorta, the smooth muscle layer from female rats produced more PGE_2 than this tissue from males, but 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ production were greater by the endothelial cell layer of male compared to female rats. Therefore, in studying the production of PGs by blood vessels from aged rats it seemed important to study the aged female as well as the aged male rat. The experiments described in this sub-section were undertaken to determine the profile of PG production by separate layers of aorta from old female rats, and to compare this profile with those obtained from young female and old male rats.

5.4.2 Methods

Female albino Wistar rats aged 2-3 months and weighing 200-250g and aged 12-14 months and weighing 330-380g were used. The old rats had been purchased from the supplier at the age of 2 months and were kept in the departmental animal house for 10-12 months. Animals were housed under controlled conditions and allowed free access to food and water. Vaginal smears were taken daily for the 2 weeks prior to the experiment and were found to show a persistent oestrous smear (i.e. persistent cornification of the vagina). Young rats exhibited regular 4 day cycles and were used on day 4. Blood pressure measurements were made daily for 2 weeks in the old rats as detailed in Section 4.1.3.

The animals were killed between 10.00h and 12.00h (n=6 in each group) and a 2.5 cm length of the thoracic aorta was removed. The three different layers of each aorta were separated, homogenised and incubated as described in Section 4.3.2. Samples in ethyl acetate were stored at -20°C until assayed by RIA for PG content as detailed in Section 2.5. The results from young and old female rats were compared using Student's t-test and significance was tested at the 5% level.

5.4.3 Results and Conclusions

Similar to the findings in male rats, the blood pressure of old female rats was significantly ($P < 0.05$) greater

than that of young female rats. Figure 5.4.1 compares PG production by homogenates of adventitia and smooth muscle from the aorta of young and old female rats. Homogenates of adventitia from young rats produced slightly more $PGF_{2\alpha}$ and PGE_2 than 6-keto-$PGF_{1\alpha}$. Homogenates of adventitia from old female rats produced mainly $PGF_{2\alpha}$ with lower amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$. The smooth muscle layer had a much greater capacity for PG synthesis than the adventitia. 6-Keto-$PGF_{1\alpha}$ was the major PG produced by young rats followed by $PGF_{2\alpha}$ and PGE_2. 6-Keto-$PGF_{1\alpha}$ was produced in greatest amounts by the smooth muscle layer of old rats with similar amounts of $PGF_{2\alpha}$ and PGE_2 formed. The amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 synthesised by the adventitia were significantly higher ($P < 0.01$) in old compared to young female rats. In the homogenates of smooth muscle however, 6-keto-$PGF_{1\alpha}$ and PGE_2 were both significantly ($P < 0.001$) greater by the smooth muscle layer of old compared to young female rats. $PGF_{2\alpha}$ production did not differ between the 2 groups.

Figure 5.4.2 shows endothelial PG production expressed as ng of PG per 2.5 cm length of aorta. 6-Keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were produced in similar amounts by the endothelium of young rats with less PGE_2. Endothelial cells from the aorta of old rats produced mainly 6-keto-$PGF_{1\alpha}$ with lower amounts of PGE_2 and $PGF_{2\alpha}$. There was no difference in the production of 6-keto-$PGF_{1\alpha}$ or $PGF_{2\alpha}$ between the two groups but there was an increased production of PGE_2 by the endothelium from old female ($P < 0.02$) compared to young female rats.

Figure 5.4.1. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by homogenates of adventitia (ADV) and smooth muscle (SM) from young (2-3 months) and old (12-14 months) female rats.

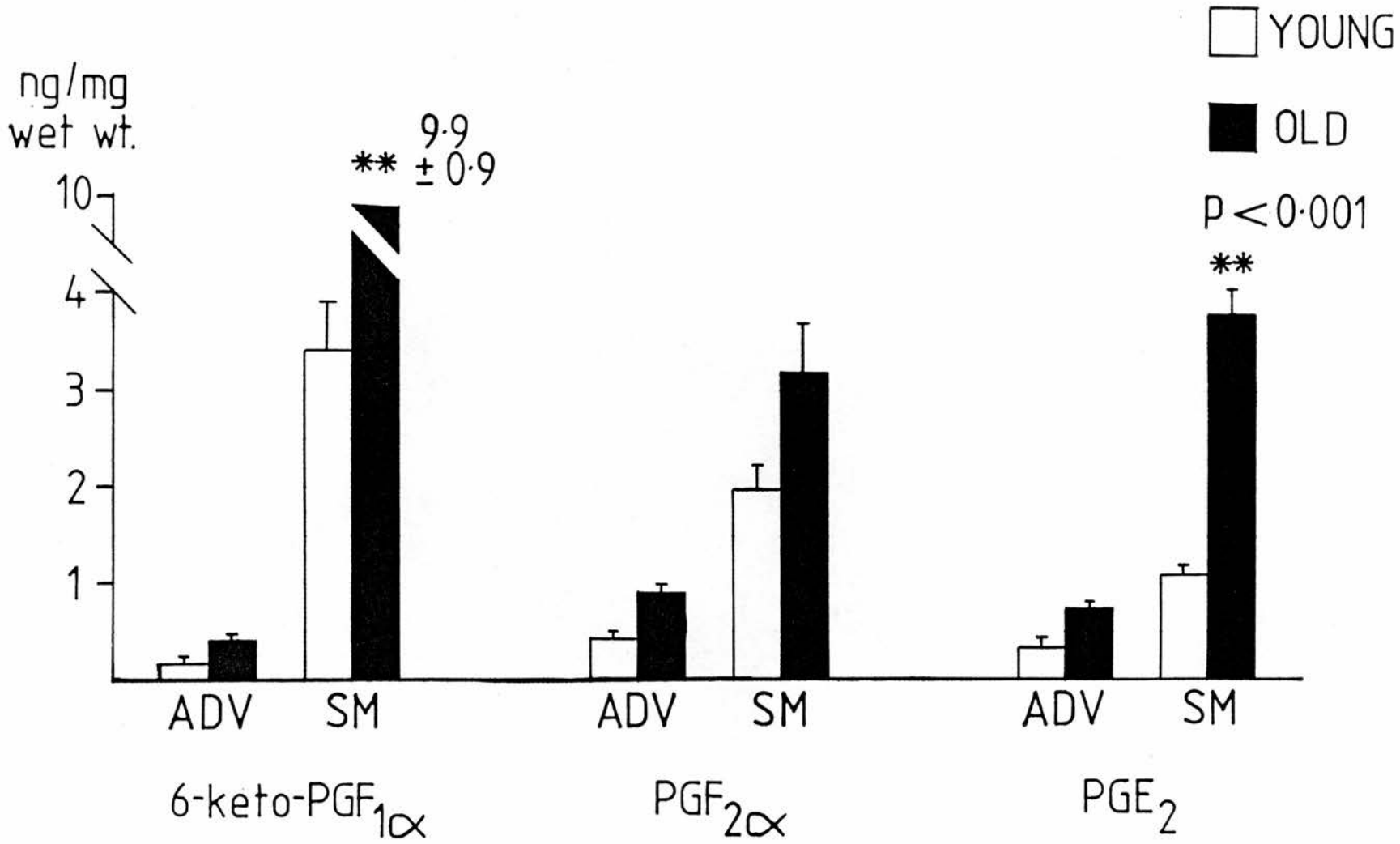


Figure 5.4.2. Mean (+/-s.e.m.,n=6) amounts of prostaglandins (PGs) synthesised by an endothelial cell suspension from a 2.5cm length of aorta from young (2-3 months) and old (12-14 months) female rats.

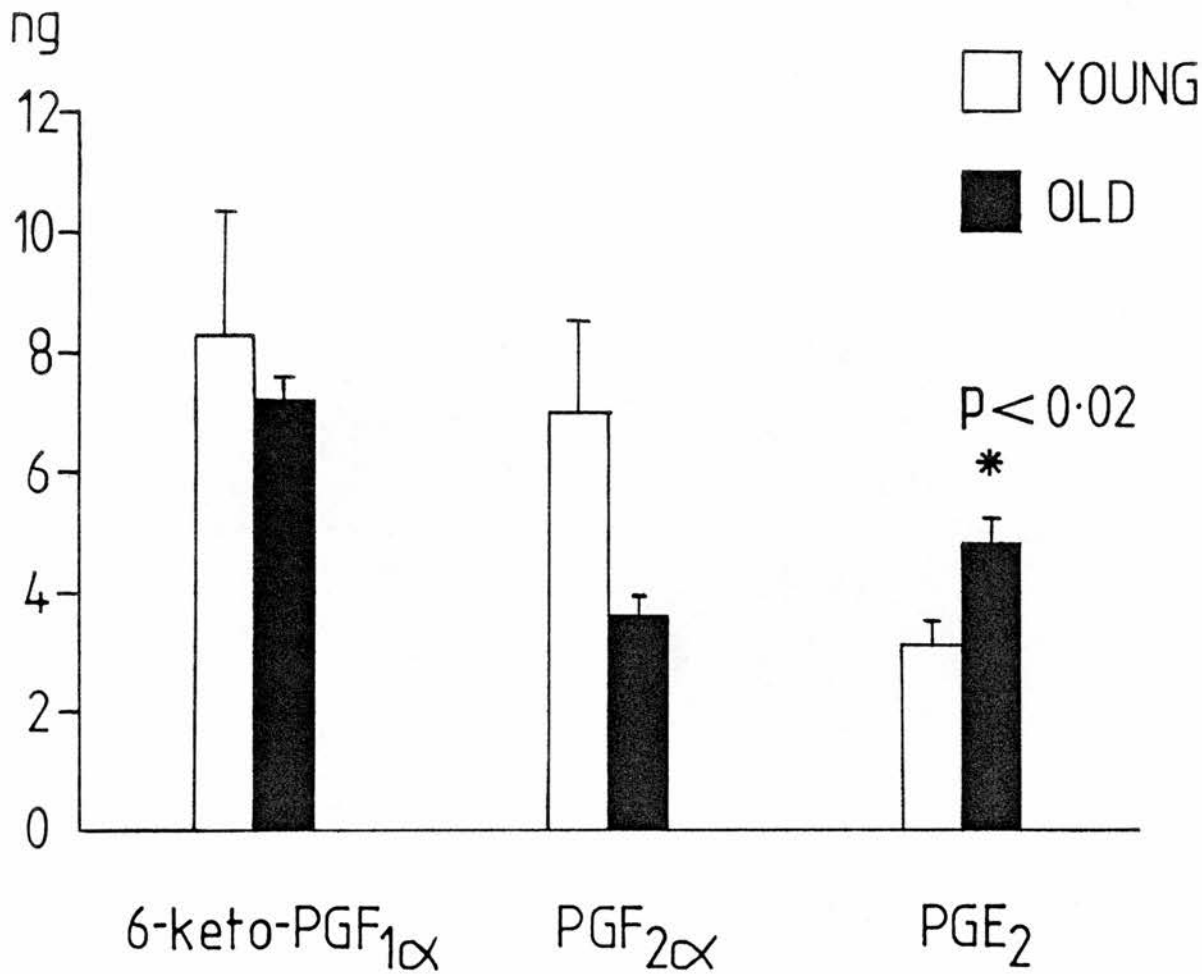


Table 5.4.1 compares the mean production of PGs by homogenates of adventitia, smooth muscle and endothelial cell layer from the aorta of old (12-14 months) male and female rats.

There was no significant difference in the production of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ by homogenates of adventitia from old male rats compared to old female rats. PGE_2 production however, was significantly ($P < 0.05$) greater by the adventitia of old female than old male rats. 6-Keto-$PGF_{1\alpha}$ production was significantly greater ($P < 0.05$) by homogenates of smooth muscle from old male compared to old female rats, whereas $PGF_{2\alpha}$ and PGE_2 production did not differ significantly between the two groups. The endothelial cell suspension from old female rats produced significantly greater amounts of 6-keto-$PGF_{1\alpha}$ and PGE_2 than the endothelium from old male rats, but there was no difference in $PGF_{2\alpha}$ production between the sexes.

Table 5.4.1.

Comparison of mean (+/-) s.e.m., n=6) production of prostaglandins (PGs) by homogenates of adventitia, smooth muscle and endothelium from aorta of old (12-14 months) male (M) and female (F) rats

	6-Keto-PGF1a		PGF2a		PGE2	
	M	F	M	F	M	F
Adventitia (ng/mg tissue)	0.34 + 0.03	0.41 + 0.05	0.83 + 0.18	0.92 + 0.08	0.45 + 0.02	0.74 + 0.07*
S. muscle (ng/mg tissue)	17.6 + 2.45	9.89 + 0.86*	4.14 + 0.40	3.20 + 0.57	2.78 + 0.40	3.78 + 0.23
Endothelium (ng/2.5cm of aorta)	4.50 + 0.40	7.20 + 0.40*	3.50 + 0.20	3.60 + 0.30	3.60 + 0.20	4.80 + 0.40*

* Significantly different from corresponding male value using Student's t-test for unpaired data, $P<0.05$.

SECTION 5.5

Measurement of unstimulated PG release from the isolated, perfused aorta of young and old female rats.

5.5.1 Introduction

It has been shown in experiments described in Section 4.7 that 6-keto-$PGF_{1\alpha}$ release from aorta is greater in males than in females. The experiments described in this sub-section were carried out to characterise the profile of basal PG release from the perfused aorta of old female rats and to compare this with the results obtained from young female rats and old male rats.

5.5.2 Methods

Female albino Wistar rats aged 2-3 months and 12-14 months and weighing 200-250g and 350-470g respectively were used. The animals were housed as before. Vaginal smears were taken daily to establish the stage of the oestrous cycle in the young rats, and to establish the presence of a persistent oestrous smear in the old rats.

Six animals from each group were killed (the young rats on Day 4 of the cycle) and the thoracic aorta was cannulated. A 2.5 cm length of aorta was removed and placed in a heated (37°C) chamber and was perfused at 5ml/min with oxygenated Krebs solution. After an equilibration period of 30 min, the perfusate was

collected for 30 min and was extracted in the same way as for homogenates as described in Section 2.4. The samples were stored in ethyl acetate until assayed for PG content as detailed in Section 2.5.

5.5.3 Results and Conclusions

Figure 5.5.1 compares the basal release of PGs from the isolated perfused aorta of young (2-3 months) and old (12-14 months) female rats. 6-Keto-$PGF_{1\alpha}$ and PGE_2 were the major PGs released by the aorta of young female rats with considerably less $PGF_{2\alpha}$ measured. The aorta from old rats released mainly 6-keto-$PGF_{1\alpha}$ followed in descending order by PGE_2 and $PGF_{2\alpha}$. The release of 6-keto-$PGF_{1\alpha}$ and PGE_2 was not significantly different between the two groups but $PGF_{2\alpha}$ release was significantly greater ($P < 0.01$) from the aortae of old compared to young female rats.

Table 5.5.1 compares the release of PGs from the isolated, perfused aorta of old (12-14 months) male and female rats.

Table 5.5.1

Comparison of mean (+/- s.e.m., n=6) release of prostaglandins from the isolated, perfused aorta of old (12-14 months) male (M) and female (F) rats.

	M	F
6-Keto-$PGF_{1\alpha}$	7.00 +/- 0.90	4.20 +/- 0.70*
$PGF_{2\alpha}$	2.80 +/- 0.70	0.90 +/- 0.07
PGE_2	2.70 +/- 0.40	2.10 +/- 0.30

* Significantly different from corresponding male value using Student's t-test for unpaired data, $P < 0.05$.

The amounts of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ released from the perfused aorta of old male rats were significantly greater ($P < 0.05$) than those from the perfused aorta of old female rats. The release of PGE_2 was not significantly different from the aorta of old males compared to females. Thus, similar to the situation observed in young animals, old males released greater amounts of 6-keto-$PGF_{1\alpha}$ from the perfused aorta than old females.

SECTION 5.6

Measurement of stimulated PG release from the isolated, perfused mesenteric arterial bed of young and old female rats.

5.6.1 Introduction

In contrast to young male rats, young females failed to show a significant stimulation of 6-keto-$PGF_{1\alpha}$ release after the 0.1 μg dose of NA and AII (see Section 4.8). Thus, it is possible that old female rats show a different response to their young counterparts with respect to NA- or AII- stimulated release of PGs in the mesenteric arterial bed.

5.6.2 Methods

Female albino Wistar rats aged 2-3 months and 12-14 months and weighing 200-250g and 350-470g respectively were used. Consecutive 4-day oestrous cycles were established in young rats and the presence of a persistent oestrous smear was confirmed in old female rats. The mesenteric arterial bed was prepared according to McGregor, (1965). This procedure is described in detail in Section 4.5.2. The vascular bed was perfused at 5 ml/min with oxygenated (95% O_2, 5% CO_2) McEwen's solution for 30 min to allow for equilibration of the tissue. NA and AII (0.1 μg and 1.0 μg) were given as a bolus injection in a random order. Samples were collected

at 1 min intervals for the 2 min before stimulation, for the 4 min immediately after stimulation and for two periods, 8 min and 12 min after stimulation. Pressor responses were recorded on a Polygraph recorder via a Statham pressure transducer. 6-Keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 content of the perfusate was measured by RIA without extraction (Section 2.5). The effects of NA and AII on PG release were tested for significance by comparing the 1 min period prior to stimulation with the two 1 min periods after stimulation using Student's t-test for paired data. Significance was tested at the 5% level.

5.6.3 Results and Conclusions

6-Keto-$PGF_{1\alpha}$ was the PG released in greatest amounts by the mesenteric bed of young female rats, with lower amounts of PGE_2 and $PGF_{2\alpha}$. 6-Keto-$PGF_{1\alpha}$ was also the major PG released by the mesenteric bed of old female rats with less PGE_2 and $PGF_{2\alpha}$. The release of all 3 PGs declined over the duration of the experiment (60 min) in both groups of rats.

The decline in the release of 6-keto-$PGF_{1\alpha}$ was significant ($P < 0.01$) in both young and old female rats. The amount of PGE_2 released by young rats declined considerably, since PGE_2 reached non-detectable levels in the perfusate of 2 out of 4 animals. The decline in the release of PGE_2 from the mesenteric bed of old rats was not significant. The amount of $PGF_{2\alpha}$ released by young females declined rapidly and reached non-detectable

levels. The release of $PGF_{2\alpha}$ from the mesenteric bed of old females also declined rapidly and reached non-detectable levels in 2 out of 4 animals. Although the initial amounts of all 3 PGs released were greater by the mesenteric vascular bed of old compared to young female rats this was not significant.

5.6.3.1 AII-stimulated PG release

AII, at both doses, produced some stimulation of 6-keto-$PGF_{1\alpha}$ release from the mesenteric vascular bed of young females but this was significant ($P < 0.05$) only for the 1.0 μg dose (Figure 5.6.1). AII had no effect on the release of $PGF_{2\alpha}$ or PGE_2. AII had a pressor effect on the mesenteric bed with the 0.1 μg dose giving a 14.7+/-6.3% increase and the 1.0 μg dose giving a 33.9+/-8.0% increase in perfusion pressure (58.3+/-2.2 mmHg) of the vascular bed. The increase in perfusion pressure was significant ($P < 0.05$) at the 1.0 μg, but not the 0.1 μg dose of AII.

In the mesenteric preparation of old rats, 0.1 μg AII failed to produce an increase, whereas 1.0 μg AII produced a significant increase ($P < 0.05$) in the release of 6-keto-$PGF_{1\alpha}$. Old female rats showed a similar response to young rats with regard to $PGF_{2\alpha}$ and PGE_2 release, with neither dose of AII producing a significant stimulation in the release of these two PGs (Tables 5.6.2 and 5.6.3). The 0.1 μg dose of AII gave a 36.1+/-8.3% rise, and the 1.0 μg dose of AII gave a 46.9+/-7.9% rise

Figure 5.6.1. Angiotensin II (AII)-stimulated release of prostaglandin (PG) I_2, measured as 6-keto-$PGF_{1\alpha}$ from the isolated, perfused mesenteric arterial bed of 4 young (2-3 months)-A, and 4 old (12-14 months) female rats-B. * compared with value immediately preceding stimulation.

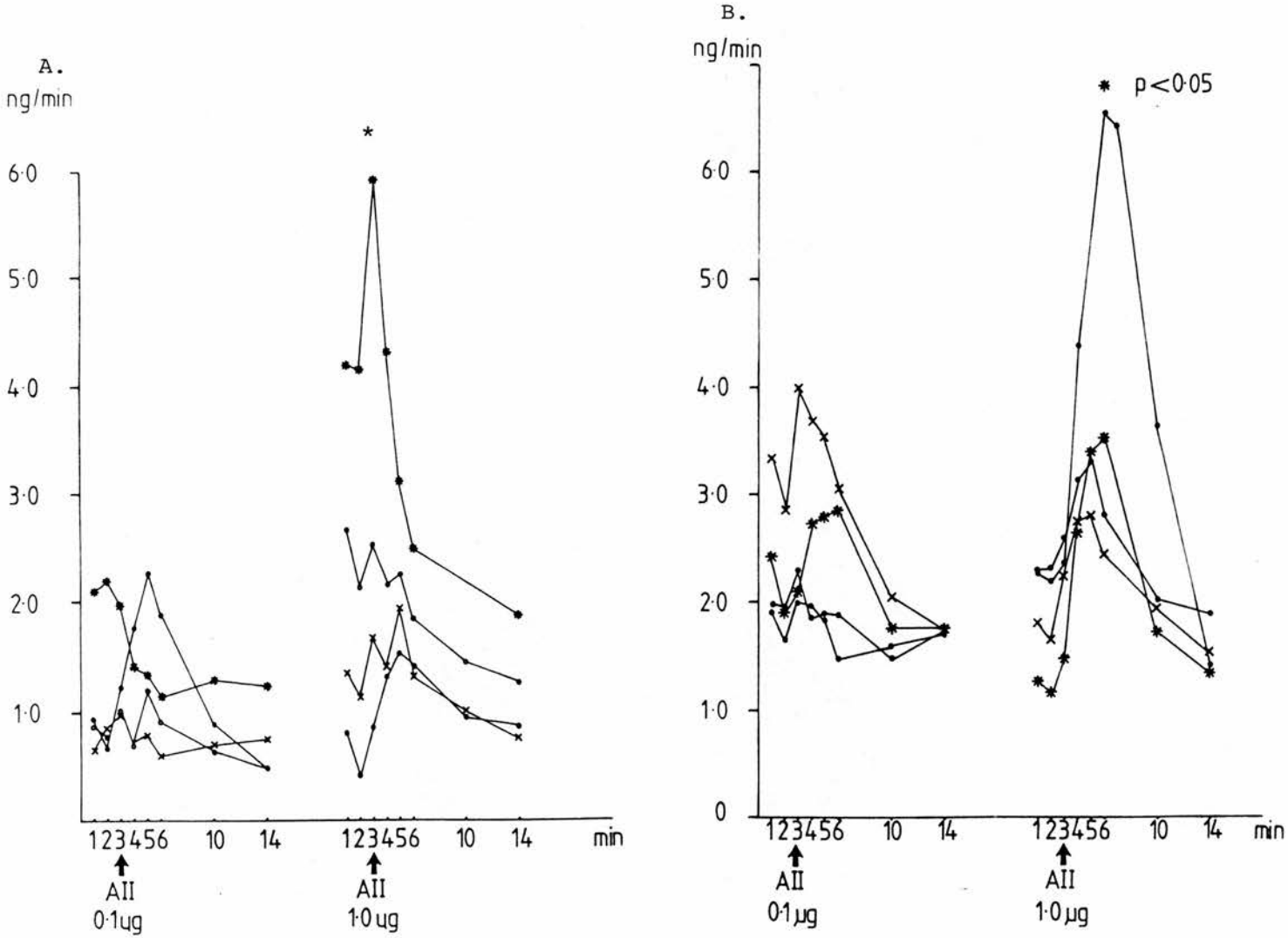


Table 5. 6.2.

Angiotensin II(AII)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated, perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) female rats (ND-< 30 pg/min)

$PGF_{2\alpha}$ release ng/min

			0.1 µg AII ↓								1.0 µg AII ↓						
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
YOUNG FEMALES	ND →								ND →							
	0.75	0.75	0.70	0.30	0.32	0.42	ND	ND	1.08	0.87	1.52	0.84	0.92	0.43	0.33	0.42
	ND →								ND →							
	ND →								0.32	0.32	0.66	0.36	0.44	0.55	0.30	0.25
OLD FEMALES	0.89	0.82	1.00	0.83	0.52	0.54	0.50	0.34	0.62	0.22	0.60	0.43	0.38	0.38	0.27	0.27
	0.39	0.47	0.60	0.44	0.36	0.34	0.52	0.54	0.85	0.73	0.86	0.67	0.35	0.35	0.54	0.51
	0.75	0.42	0.55	0.42	0.57	0.71	0.58	0.61	0.42	0.54	0.28	0.60	0.30	0.96	0.34	0.24
	1.21	1.04	1.12	0.90	0.94	0.83	1.06	0.72	1.20	1.14	0.97	0.71	1.14	1.22	1.44	1.18

Table 5.6.3.

Angiotensin II(AII)-stimulated release of prostaglandin (PG) E_2 from the isolated, perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) female rats (ND-< 30 pg/min)

	PGE_2 release ng/min															
			0.1 µg AII ↓								1.0 µg AII ↓					
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
	ND →								ND →							
YOUNG FEMALES	0.70	1.01	0.61	ND	ND	0.35	0.46	0.52	1.18	0.86	1.46	0.72	0.78	1.82	1.55	1.26
	0.30	0.42	0.40	0.32	0.40	0.51	0.38	0.22	0.16	ND	0.24	0.26	0.50	ND	0.16	0.30
	ND	ND	ND	0.21	0.40	0.34	0.23	0.52	0.38	0.35	0.51	0.50	0.64	0.74	0.23	0.24
OLD FEMALES	0.50	0.54	0.68	0.51	0.30	0.39	0.32	ND	0.39	0.42	0.28	0.20	0.36	0.49	0.34	0.56
	0.38	0.34	0.47	0.26	0.25	0.37	0.26	0.22	0.74	0.56	0.44	0.48	0.51	0.30	0.35	0.34
	1.50	1.04	1.30	1.10	1.06	0.76	0.46	0.96	0.57	1.06	0.46	0.96	0.91	1.13	0.76	0.43
	0.84	0.92	0.60	0.66	0.65	0.29	0.55	0.61	0.62	0.71	0.80	0.52	0.62	0.75	0.60	0.66

in basal perfusion pressure of the mesenteric bed. Both doses of AII produced a significant ($P < 0.01$) increase in perfusion pressure. There was no significant difference in the pressor response to AII in the mesenteric bed of young compared to old female rats.

5.6.3.2 NA-stimulated PG release

NA at the 1.0 μg, but not the 0.1 μg dose, produced a significant increase in the release of 6-keto-$PGF_{1\alpha}$ from the mesenteric bed of young female rats (Figure 5.6.2). Similarly, there was no significant effect of NA on the release of either $PGF_{2\alpha}$ or PGE_2. NA produced a rise in basal perfusion pressure of 40.8+/-4.7% at the 0.1 μg dose and a 150.4+/-3.2% increase at the 1.0 μg dose of NA. The rise in perfusion pressure was significant ($P < 0.05$) at both doses of NA.

In the mesenteric vascular bed of old female rats, NA at the 0.1 μg dose failed to have any significant effect on the release of 6-keto-$PGF_{1\alpha}$. The 1.0 μg dose however, produced a significant increase ($P < 0.05$) in the amount of 6-keto-$PGF_{1\alpha}$ released. There was no significant effect of NA on the release of $PGF_{2\alpha}$ at either dose tested (Table 5.6.4). The 0.1 μg dose of NA had no effect on the release of PGE_2 and although there was some stimulation of PGE_2 release at the higher dose, this was not significant (Table 5.6.5). Noradrenaline (0.1 μg), caused a 86.5+/-10.1% increase in basal perfusion

Figure 5.6.2. Noradrenaline (NA)-stimulated release of prostaglandin (PG) I_2, measured as 6-keto-$PGF_{1\alpha}$ from the isolated, perfused mesenteric arterial bed of 4 young (2-3 months)-A, and 4 old (12-14 months) female rats-B. * compared with value immediately preceding stimulation.

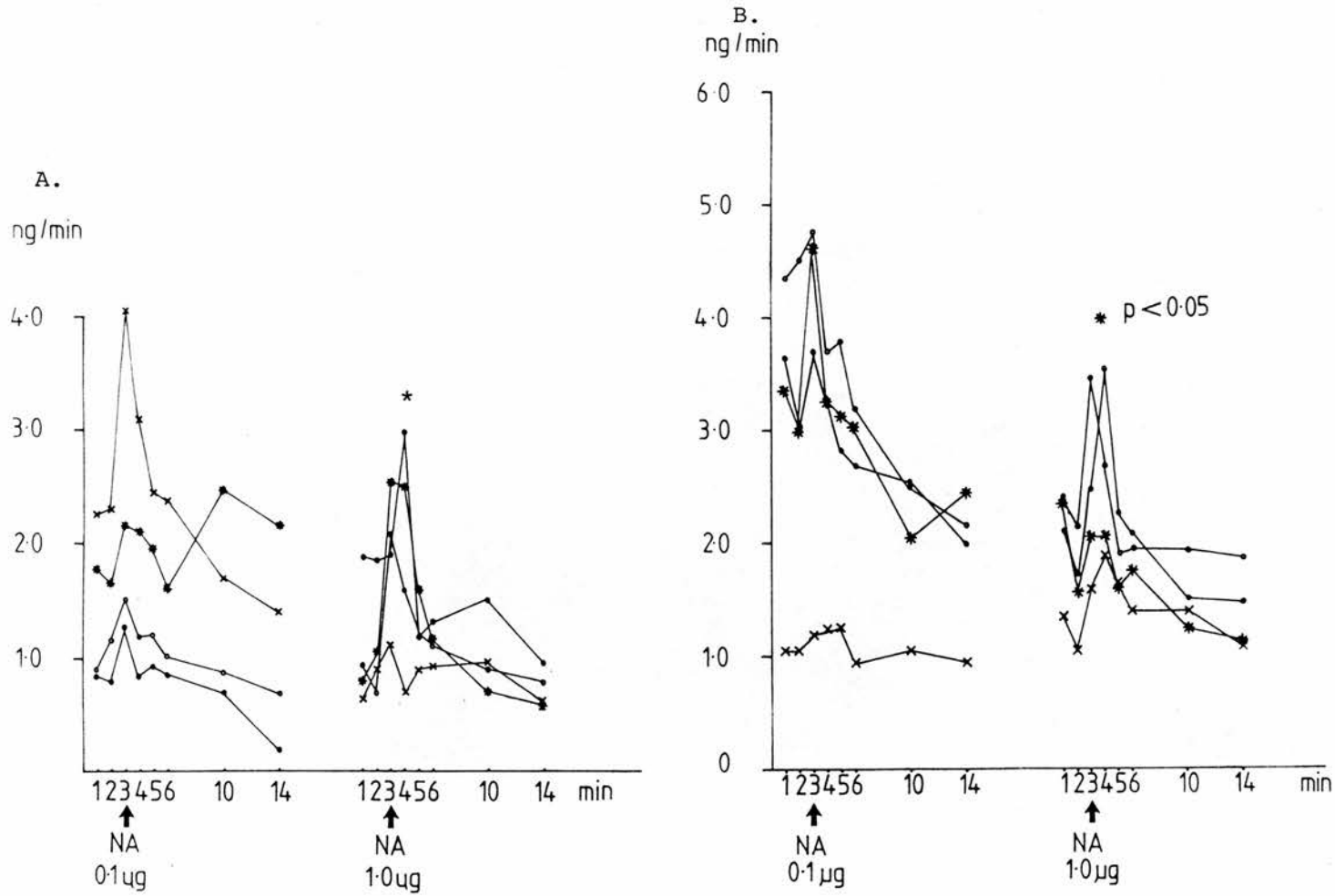


Table 5.6.4.

Noradrenaline (NA)-stimulated release of prostaglandin (PG) $F_{2\alpha}$ from the isolated, perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) female rats (ND-< 30 pg/min)

	$PGF_{2\alpha}$ release ng/min															
			0.1 µg NA ↓								1.0 µg NA ↓					
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
YOUNG FEMALES	ND →								ND →							
	0.64	0.53	0.44	0.40	0.70	0.68	0.35	0.75	0.19	0.86	0.85	0.33	0.29	0.30	0.18	0.43
	ND →								0.36	0.42	0.45	0.34	0.50	0.45	0.50	0.35
	ND →								0.16	0.62	0.22	0.22	0.27	0.28	0.22	0.64
OLD FEMALES	ND →								0.20	ND	ND	0.30	0.46	ND	0.32	ND
	1.34	1.26	1.31	1.06	0.82	0.95	1.01	0.60	0.39	0.58	0.95	1.17	0.74	0.30	0.30	0.53
	0.60	0.70	0.96	0.93	1.00	0.81	0.66	0.90	0.51	0.28	0.44	0.48	0.28	0.34	0.38	0.28
	1.44	1.68	1.50	1.03	1.25	1.48	1.01	1.01	1.12	1.16	1.23	0.94	0.74	0.54	1.46	1.34

Table 5.6.5.

Noradrenaline (NA)-stimulated release of prostaglandin (PG) E_2 from the isolated, perfused mesenteric arterial bed of young (2-3 months) and old (12-14 months) female rats (ND-< 30 pg/min)

	PGE_2 release ng/min															
			0.1 µg NA ↓								1.0 µg NA ↓					
TIME : MIN	-1	-2	1	2	3	4	10	14	-1	-2	1	2	3	4	10	14
YOUNG FEMALES	ND								ND							
	1.28	0.79	0.22	0.65	0.44	1.71	1.25	1.15	0.38	0.22	ND	ND	0.62	ND	ND	ND
	0.26	0.34	0.24	0.56	0.24	0.24	0.52	0.48	0.58	0.54	0.61	0.59	0.67	0.74	0.67	0.56
	0.16	0.19	0.75	0.48	ND	ND	ND	ND	ND	0.43	0.51	0.74	ND	0.21	0.35	0.39
OLD FEMALES	ND	ND	ND	0.38	0.28	0.38	0.29	0.48	0.39	0.28	0.54	0.41	0.28	0.35	0.47	0.30
	0.72	0.67	1.00	0.60	0.62	0.50	0.84	0.57	0.32	0.54	0.81	0.87	0.34	0.57	0.40	0.37
	1.75	1.44	1.29	1.04	0.90	0.80	0.77	1.32	0.96	0.64	1.27	1.22	1.00	0.70	0.60	0.59
	0.84	0.97	1.04	1.60	1.06	0.93	0.97	0.50	0.70	0.92	0.65	0.86	0.78	0.61	0.45	0.48

pressure and the 1.0 μg dose produced a 259.4+/- 11.8% increase in the perfusion pressure of the mesenteric bed. The increase in perfusion pressure was significant at the 0.1 μg ($P < 0.05$) and at the 1.0 μg ($P < 0.01$) dose of NA. There was a significantly greater ($P < 0.05$) pressor response to NA in the mesenteric bed of old compared to young female rats.

SECTION 5.7

A comparison of the effects of angiotensin II (AII) and noradrenaline (NA) on PG release from the perfused mesenteric arterial bed of old (12-14 months) male and female rats.

Table 5.7.1 compares the 6-keto-$PGF_{1\alpha}$ releasing abilities of NA and AII in old male compared to old female rats.

Table 5.7.1

Comparison of the ability of noradrenaline (NA) and angiotensin II AII) to cause a significant ($P < 0.05$) or non-significant (NS) release of 6-keto-$PGF_{1\alpha}$ from the isolated perfused mesenteric bed of old male and female rats.

	NA		AII	
	0.1 μg	1.0 μg	0.1 μg	1.0 μg
MALE	$P< 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
FEMALE	NS	$P < 0.05$	NS	$P < 0.05$

Old male and female rats were similar in their responses to the higher (1.0 μg) dose of NA and AII, but old female rats failed to show a significant increase in 6-keto-$PGF_{1\alpha}$ release at the low (0.1 μg) dose of either NA or AII. There was no effect of either NA or AII on $PGF_{2\alpha}$ and PGE_2 release in both male and female rats.

5.8 Discussion

In conclusion, a number of differences in PG production and output were observed in the blood vessels of old male and female rats compared to young male and female rats. Specifically, an increase in age produced a marked stimulation of 6-keto-$PGF_{1\alpha}$ production by homogenates of aortic smooth muscle in both male and female rats, with the greater stimulation being found in male rats. Conversely, there was a significant depression in 6-keto-$PGF_{1\alpha}$ production by aortic endothelial cells of old male, but not old female rats. However, these differences in PG production were not reflected in a significant change in 6-keto-$PGF_{1\alpha}$ output from the isolated perfused aorta with age, although 6-keto-$PGF_{1\alpha}$ output did tend to be higher in the older rats of both sexes. There was a sex difference in PG output from the aorta, 6-keto-$PGF_{1\alpha}$ output being significantly lower in young and old female rats, than in young and old male rats, respectively. In the mesenteric arterial bed, the basal release of 6-keto-$PGF_{1\alpha}$ was higher in old male and female rats than in young male and female rats, and 6-keto-$PGF_{1\alpha}$ release was higher in old male rats than in old female rats. The increases in 6-keto-$PGF_{1\alpha}$ output stimulated by NA and AII were not attenuated with an increase in age in either male or female rats.

Considering the other 2 PGs, homogenates of aortic smooth muscle again showed the greatest changes, with $PGF_{2\alpha}$ and PGE_2 production increasing with age in male rats and PGE_2

production increasing with age in female rats. PGE_2 production by aortic endothelial cells increased with age in old female, but not old male rats. The output of PGE_2 from the perfused aorta did not change significantly with age, but $PGF_{2\alpha}$ output was significantly increased from the aorta of old male and female rats. Moreover, $PGF_{2\alpha}$ was readily detectable in perfusates of the mesenteric arterial bed of old male and female rats, whereas the amounts of $PGF_{2\alpha}$ were below the detection limit of the assay in young male and female rats. Thus, there was an overall increase in the output of $PGF_{2\alpha}$ from the vasculature of the rat (both sexes) with an increase in age. This finding is of interest in view of the action of $PGF_{2\alpha}$ as a vasoconstrictor, but also as a potentiator of the responses to sympathetic nerve stimulation in blood vessels (Brody and Kadowitz, 1974). Indeed, this study has shown that the pressor response to NA but not to AII, was significantly greater in old than in young, male and female rats. Whether this increase in vascular $PGF_{2\alpha}$ production is involved in the increase in blood pressure of these rats with age requires further investigation. Obviously, this study is using an animal model to look at the effects of increasing age on vascular PG production and the situation in humans may be different. Consequently, the experiments described in the next section aimed to investigate the profile of PG production by aorta and saphenous vein of human subjects and to determine if this profile was affected by age, sex or drug treatment.

SECTION 6.0

Measurement of the PG synthetic capacity of homogenates of aorta and saphenous vein and the release of PGs from rings of saphenous vein from male and female human subjects : effects of age, sex and drug treatment.

Introduction

Following the discovery of PGI_2 production by rabbit blood vessels it was established that human mesenteric artery and vein also produce this PG (Moncada et al., 1977). Rings of human umbilical artery (Ritter, Ongari, Barrow, Orchard, Blair and Lewis, 1982) and rings of forearm vein obtained by biopsy have also been shown to release PGI_2 (Preston, Greaves, Jackson and Stoddard, 1982). For obvious reasons it is difficult to obtain human vascular tissue for studies investigating PG production and release, so umbilical artery or umbilical vein endothelial cells in culture are commonly used in studies of human vascular PG production. Gimbrone and Alexander (1975) showed that cultured umbilical vein endothelial cells release PGE into the medium and that this release is stimulated by AII. In 1977 Weksler, Marcus and Jaffe demonstrated inhibition of platelet aggregation by intact cultured umbilical vein endothelial cells and these cells converted [^{3}H] AA to PGI_2 and PGE_2. Baenzinger, Dillinger and Majerus (1977) demonstrated that human aortic smooth muscle cells in culture produce

PGI_2. Later studies have shown that a number of agents will stimulate PGI_2 production by cultured umbilical vein endothelial cells e.g. AA, histamine, thrombin and A23187 but that unstimulated umbilical vein endothelial cells do not produce detectable amounts of PGI_2 (Baenzinger, Force and Becherer, 1980; Baenzinger, Fogerty, Mertz and Chernuta, 1981; Weksler, Ley and Jaffe, 1978). Another study by Alhenc-Gelas, Tsai, Callaghan, Campbell and Johnson, (1982) has shown that arterial and venous umbilical endothelial cells in culture synthesise $PGF_{2\alpha}$ and PGE_2 as well as PGI_2 and that histamine stimulates not only PGI_2 release but also $PGF_{2\alpha}$ and PGE_2 release from venous cells. In addition, bradykinin enhanced the release of PGE_2 (as well as PGI_2) in arterial cells. However, the use of umbilical vascular tissue to demonstrate PG production has been criticised since the stimulation of PGI_2 formation in such tissues by various agents may not neccessarily represent the situation in other parts of the vasculature.

As PGI_2 is not extensively metabolised by the lung and experiments using bio-assay suggested that PGI_2 produced by the lung (and possibly by other tissues) acted as a circulating platelet inhibitory factor and a vasodepressor agent in rabbits and cats (Moncada et al., 1978; Gryglewski et al., 1978). Studies in humans in which trans-pulmonary measurements of plasma 6-keto-$PGF_{1\alpha}$ concentrations were made by GC-MS in patients undergoing cardiac catheterisation (Hensby et al., 1979) suggested that PGI_2 was released from the lung at a rate

(5ng/kg/min) which is sufficient to inhibit platelet aggregation *in vitro*, as established when synthetic PGI_2 was infused into human volunteers (Fitzgerald, Friedman, Miyamori, O'Grady and Lewis, 1979). However, evidence now suggests that the rate of secretion of PGI_2 into the circulation of human volunteers is very much lower, approximately 0.09 ng/kg/min (Fitzgerald et al., 1981). Other studies have substantiated this finding using RIA (Forder and Carey, 1982) where venous plasma levels of approximately 7 pg/ml were reported, and capillary column gas chromatography with negative ion mass spectrometry where plasma levels of 6-keto-$PGF_{1\alpha}$ were found to be less than 3 pg/ml (Blair et al., 1982).

Studies yielding interesting findings on 6-keto-$PGF_{1\alpha}$ levels under different conditions and pathological states have been carried out, but the above findings must be considered when discussing these results, and in a number of cases the validity of the absolute concentrations of 6-keto-$PGF_{1\alpha}$ quoted, must be questioned. Hensby et al. (1981) measured venous plasma 6-keto-$PGF_{1\alpha}$ by GC-MS and found no significant difference between normal males and females. Another study by Ylikorkala, Osterman, Linden and Viinika (1982) found that plasma 6-keto-$PGF_{1\alpha}$ (as measured by RIA) was higher in subjects of 10-20 years than subjects of 21-30 or 51-70 years. The levels of 6-keto-$PGF_{1\alpha}$ in subjects of 71-90 years did not differ significantly from those under 20 years and the levels in women over 70 years were significantly higher than those in men of the same age. Plasma 6-keto-$PGF_{1\alpha}$ was found to

be significantly lower in women on combined oestrogen and progestagen oral contraceptives than in controls but women on a progestagen only pill had levels of 6-keto-$PGF_{1\alpha}$ in the normal range (Ylikorkala, Puolakka and Viinika, 1982). Using an alternative approach Nordoy, Svensson, Haycraft, Hoak and Wiebe (1978) observed that spontaneous platelet aggregation was commonly found in women (21-31 years) using combined oral contraceptives, post-menopausal women (45-66 years) and men in the 48-71 age group. The inhibitory effect of human endothelial cells on ADP-induced aggregation was significantly reduced in young women taking oral contraceptives and in post-menopausal women. Also, the inhibitory effect of PGI_2 on ADP-induced aggregation was less in all groups of females compared to males. Thus, a number of differences in plasma levels of 6-keto-$PGF_{1\alpha}$ have been observed depending on age, sex and whether the women have been taking oral contraceptives or not.

A number of studies have been carried out to determine if levels of 6-keto-$PGF_{1\alpha}$ differ in normotensive compared to essentially hypertensive subjects. Grose, Lebel and Gbeassor (1980), observed a diminished excretion of 6-keto-$PGF_{1\alpha}$ in the urine of patients with essential hypertension compared to controls. In agreement with these findings plasma 6-keto-$PGF_{1\alpha}$ levels (as measured by RIA) were found to be significantly reduced in hypertensive compared to normotensive subjects but, during an infusion of NA, there was a greater increase in 6-keto-$PGF_{1\alpha}$ in the plasma of hypertensive than

normotensive subjects (Ishii, Uehara, Hirata, Atarashi, Ikeda, Sugimoto, Takeda and Murao, 1982). However, Friedman, Webster, Hensby and Lewis (1981) found that, although plasma 6-keto-$PGF_{1\alpha}$ was slightly elevated in patients with essential hypertension, it was not significantly elevated compared to age-matched controls. The hypertensive patients in the former study were older than their normotensive controls and this may have affected the results.

The present study aimed to characterise the profile of PG production by homogenates of aorta and saphenous vein, and the release of PGs from rings of saphenous vein from a large number of male and female subjects. These subjects were patients undergoing surgery for coronary heart disease and despite the limitations imposed by such a population group the effects of a number of parameters (i.e. age, sex, smoking habits and drug regime) on vascular PG production has been investigated.

6.1.1 Patient Characteristics

A total of 127 patients were studied, 88 males and 39 females. The age range for the males was 36-79 years (mean +/- s.e.m.; 54.4 +/-0.9) and for the females was 30-76 years (mean +/- s.e.m.; 54.7 +/- 2.5). Of the 88 males; 24 were smokers, 31 had stopped smoking for some time prior to the operation and 33 were non-smokers. Of the 39 females; 10 were smokers, 19 had stopped smoking

Table 6.1.1.

Numbers of men and women receiving various drug treatments and their smoking habits according to age groups

Age (years)	Total Number	Drug Treatment					Smoking Habit		
		A	B	C	D	E	NS	S	XS
					MEN				
31-40	3	2	3	1	0	2	0	2	1
41-50	26	16	19	3	3	16	7	10	9
51-60	37	24	28	4	5	25	14	11	12
61-70	20	11	14	4	5	2	11	1	8
71-80	2	1	1	1	1	2	1	0	1
TOTAL	88	54	65	13	14	47	33	24	31
					WOMEN				
31-40	2	2	1	1	0	1	0	2	0
41-50	6	5	3	0	1	4	0	1	5
51-60	13	10	9	6	3	6	2	4	7
61-70	16	9	15	4	5	9	6	7	3
71-80	2	0	2	0	0	1	2	0	0
TOTAL	39	26	30	11	9	21	10	14	15

A = β-adrenoceptor blocking drugs; B = Nitrate vasodilators; C = Thiazide diuretics; D = Loop diuretics; E = Ca^{2+} channel blocking drungs; NS = Non-smokers; S = Smokers; XS = Ex-smokers

some time prior to the operation and 10 were non-smokers. All of the patients were receiving a range of medications and the major drugs being taken, age groupings and smoking habits are detailed in Table 6.1.1.

A large percentage of men (61.4%) and women (66.7%) were taking a β-adrenoreceptor blocking drug, and a large percentage (men 73.8%, women 76.9%) were also taking a nitrate drug. A greater proportion of women were taking a loop diuretic compared to men, 23.1 % and 15.9 % respectively. A greater percentage of men (83%) were taking a Ca^{2+} antagonist compared to women (46.2%) and a greater percentage of women (33.3%) were taking a thiazide diuretic compared to men (12.5%).

6.1.2 Effects of age and sex on PG production by aorta and saphenous vein of male and female subjects.

6.1.2.1 Introduction

The 127 patients were divided into groups according to age and sex. Thus males and females were divided into 5 age groups; 31-40, 41-50, 51-60, 61-70 and 71-80 years. The majority of male patients (73%) were aged 41 to 60 years whereas the majority of female patients (74%) were aged 51-70 years. Due to the nature of the operation there are no patients under the age of 30 years and very few in the 31-40 age group, therefore the discussion of the effects of age on vascular PG production will be

limited to subjects of middle age and older. Also, the well-documented difference in the incidence of cardiovascular disease between men and women is reflected in this sample group, since only 31% of the total number of patients were female.

6.1.2.2 Methods

Vascular tissue was obtained from patients undergoing coronary artery by-pass surgery. From each patient an excess segment of saphenous vein and the small piece of aorta punched out during the joining of the section of saphenous vein to the aorta were placed in Krebs solution by the operating theatre nurse. The tissue was then collected as soon as possible from the operating theatre. The aorta and segment of saphenous vein were blotted dry, weighed, and then homogenised separately in 5ml Krebs solution. The homogeniser was washed with 5ml Krebs solution and the homogenate and washings were added to 25ml conical flasks. The homogenates were incubated with 2 μg/ml AA, for 60 min at 37°C, in an atmosphere of 95% O_2, 5% CO_2. The incubates were then extracted with ethyl acetate as described in Section 2.4. The dried extracts were redissolved in 4 ml ethyl acetate and stored at -20°C until assayed for PG content by RIA as detailed in Section 2.5. If sufficient saphenous vein tissue was obtained, the piece of tissue remaining after using a segment for homogenisation was cut into 2mm rings and was incubated in 5ml Krebs solution. The PGs present in the incubation fluid from the rings were extracted with ethyl

acetate and assayed for PG content as described in Sections 2.4 and 2.5 respectively. The tissue samples were collected from the operating theatre by Mr. Ian Ansell of the department of Clinical Surgery, Royal Infirmary and coded by him before being passed on to the Department of Pharmacology. All of the patient details were collated by Mr.Ansell and were not consulted until after the RIAs had been completed. The results obtained were analysed with respect to age and sex, the effects of cigarette smoking, and the effects of various drug treatments, as follows:-

6.1.2.3 Results

Table 6.1.2 shows the mean (+/- s.e.m.) amounts of PGs produced by homogenates of aorta and saphenous vein of male and female subjects respectively, according to age.

6.1.2.4 Conclusions

It can be seen from Table 6.1.2 that homogenates of aorta from male subjects produced similar amounts of the three PGs. Homogenates of saphenous vein however, produced mainly 6-keto-$PGF_{1\alpha}$ with lesser amounts of $PGF_{2\alpha}$ and PGE_2. This profile of PG production was similar in all of the age groups studied. Homogenates of aorta and saphenous vein produced similar amounts of 6-keto-$PGF_{1\alpha}$, but aortic $PGF_{2\alpha}$ and PGE_2 production were considerably greater than that of the saphenous vein. Again this was true for all age groups studied. There were no significant differences in PG production by homogenates

Table 6.1.2. Mean (± s.e. mean) amounts of prostaglandins (PGs) produced by homogenates of aorta (A) and saphenous vein (V) of men and women according to age groups

		PROSTAGLANDIN PRODUCTION (ng/mg tissue)				
Age (years)		31-40	41-50	51-60	61-70	71-80
		MEN				
n =		3	26	38	19	2
6-Keto-$PGF_{1\alpha}$	A	10.0 ± 2.3	12.7 ± 1.9	12.3 ± 1.6	15.0 ± 3.0	4.8
	V	11.9 ± 5.2	15.0 ± 1.5	16.4 ± 1.9	17.8 ± 3.0	10.8
$PGF_{2\alpha}$	A	17.3 ± 7.0	21.0 ± 4.9	13.6 ± 1.5	12.4 ± 1.8	4.1
	V	5.7 ± 1.8	7.7 ± 1.5	7.1 ± 0.8	7.6 ± 0.9	5.0
PGE_2	A	18.0 ± 8.9	16.6 ± 3.5	11.7 ± 1.2	11.4 ± 1.9	4.5
	V	4.1 ± 0.8	4.2 ± 0.6	4.1 ± 0.4	4.4 ± 0.9	3.2
		WOMEN				
n =		2	6	13	16	2
6-keto-$PGF_{1\alpha}$	A	7.2	13.1 ± 3.4	12.2 ± 2.1	19.7 ± 3.3	6.7
	V	6.8	6.1 ± 1.6*	16.6 ± 2.3^{+}	7.4 ± 0.7*	25.2
$PGF_{2\alpha}$	A	4.0	11.2 ± 3.0	19.0 ± 4.6	10.3 ± 1.4	14.6
	V	10.1	17.9 ± 4.2*	8.0 ± 0.9^{+}	16.3 ± 2.3*	7.9
PGE_2	A	3.6	10.2 ± 2.1	10.6 ± 1.4	15.7 ± 3.1	14.2
	V	12.7	3.7 ± 1.3	4.4 ± 0.4	4.4 ± 0.7	4.4

$^{+}$ Significantly different ($P < 0.01$) from corresponding value in female groups of 41-50 and 61-70 years.
* Significantly different ($P < 0.01$) from corresponding value in male group of same age.

of aorta and vein when comparing the age groups although there did appear to be a trend towards increased production of 6-keto-$PGF_{1\alpha}$ by aorta and vein with increased age. There was also a trend towards decreased production of both $PGF_{2\alpha}$ and PGE_2 by the aorta, but not by the vein with increasing age. The profile of PG production by blood vessels of female subjects was slightly different to that of the males (Table 6.1.2).

As was observed for males, the aorta from females produced similar amounts of the three PGs. The profile of PG production by the saphenous vein of females was more complex with differences being found in different age groups. In the 41-50 age group, $PGF_{2\alpha}$ was the major PG produced with lesser amounts of 6-keto-$PGF_{1\alpha}$ and PGE_2; in the 51-60 age group, 6-keto-$PGF_{1\alpha}$ was the major PG produced with lesser amounts of $PGF_{2\alpha}$ and PGE_2; and in the 61-70 age group, $PGF_{2\alpha}$ was the major PG produced with lesser amounts of 6-keto-$PGF_{1\alpha}$ and PGE_2. These differences in the profile of PG production were reflected by significant differences in PG production with age; the veins of women aged 51-60 produced significantly greater ($P<0.01$) amounts of 6-keto-$PGF_{1\alpha}$ compared to the veins of women aged 41-50 or 61-70 years of age. The veins of women in the 51-60 age group also produced significantly less ($P<0.01$) $PGF_{2\alpha}$ than the veins of women in the 41-50 and 61-70 age groups. There was a change in the 6-keto-$PGF_{1\alpha}$ to $PGF_{2\alpha}$ ratio from 2.07 : 1.0 in the 51-60 age group to 0.34 : 1.0 and 0.45 : 1.0 in the 41-50 and 61-70 age groups respectively. PGE_2

production did not vary significantly with age. There were no significant differences in the production of the 3 PGs by homogenates of aorta with age.

Comparing males and females in the same age group there was no significant sex difference in the aortic production of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ or PGE_2. There were significant sex differences however, in the venous production of 6-keto-$PGF_{1\alpha}$, with females in the 41-50 and 61-70 age groups producing smaller ($P<0.01$) amounts of 6-keto-$PGF_{1\alpha}$ from homogenates of saphenous vein than males in the same age group. But, females in the 41-50 and 61-70 age groups produced significantly ($P<0.01$) more $PGF_{2\alpha}$ from venous homogenates than males in the same age groups. There was no significant difference in the production of PGE_2 by veins of males compared to females.

6.1.3 Measurement of the basal release of PGs from rings of saphenous vein from male and female patients.

6.1.3.1 Introduction

The experiments described in the previous sub-section give details of PG production by homogenates of aorta and vein incubated with exogenous AA. Experiments described here aimed to characterise the basal release of PGs from rings of saphenous vein in the absence of stimulation with AA and to determine if there were any age or sex differences in PG release. A total of 48 males and 16 females were studied in this way.

6.1.3.2 Results

Tables 6.1.4 and 6.1.5 show the mean (+/-s.e.m.) amounts of PGs released from rings of saphenous vein from male and female subjects divided according to age.

Table 6.1.4

Mean (+/-s.e.m.) release of prostaglandins (PGs) from rings of saphenous vein from male subjects aged 35-79 years.

	Prostaglandin output (ng/mg wet wt/30 min)				
AGE GROUP	31-40	41-50	51-60	61-70	71-80
n	(2)	(11)	(20)	(16)	(0)
6-keto-$PGF_{1\alpha}$	1.9	4.5+/-1.2	4.9+/-1.0	3.8+/-0.5	-
$PGF_{2\alpha}$	1.6	2.5+/-0.4	2.8+/-0.5	1.9+/-0.3	-
PGE_2	1.3	2.7+/-0.6*	2.2+/-0.3	1.5+/-0.2*	-

* Significantly ($P<0.05$) lower than males in the 41-50 age group using Student's t-test.

Table 6.1.5

Mean (+/-s.e.m.) release of prostaglandins (PGs) from rings of saphenous vein from female subjects aged 30-76 years.

	Prostaglandin output (ng/mg wet wt/30 min)				
AGE GROUP	31-40	41-50	51-60	61-70	71-80
n	(1)	(2)	(6)	(6)	(1)
6-keto-$PGF_{1\alpha}$	3.8	5.5	4.6+/-0.8	5.2+/-1.3	4.7
$PGF_{2\alpha}$	0.7	3.3	1.7+/-0.2	4.0+/-1.3#	2.9
PGE_2	1.9	1.8	1.6+/-0.2	2.0+/-0.2	2.0

Significantly ($P<0.05$) different from corresponding male value using Student's t-test.

6.1.3.3 Conclusions

6-Keto-$PGF_{1\alpha}$ was the major PG released in all age groups of male subjects followed by lesser and approximately equal amounts of $PGF_{2\alpha}$ and PGE_2. (Table 6.1.4). There was a decline in the release of all 3 PGs in the 61-70 compared to the 41-50 years age group; this was not significant for 6-keto-$PGF_{1\alpha}$ or $PGF_{2\alpha}$ but was significant ($P<0.05$) for PGE_2. 6-Keto-$PGF_{1\alpha}$ was also the major PG released by saphenous vein of females with lesser and similar amounts of $PGF_{2\alpha}$ and PGE_2 (Table 6.1.5). Because of the small number of female subjects it was not possible to determine if there were any age differences

in PG production. The only sex difference apparent in the release of PGs was in the 61-70 years age group where the vein from females released significantly ($P<0.05$) greater amounts of $PGF_{2\alpha}$ than the males.

Section 6.2.1

The effects of cigarette smoking on PG production by aorta and saphenous vein of male and female subjects.

6.2.1.1 Introduction

Smoking is considered to be one of the major risk factors for coronary heart disease (US Report to the Surgeon General on Smoking and Health, 1963; Kannel and Thom, 1979). Several studies have been carried out to investigate the effects of cigarette smoke on the blood vessel wall and also on vascular PG production. Pittilo et al., (1982) exposed rats to graded doses of fresh cigarette smoke (5 days per week for 25 days) and examined the aortic endothelium by scanning electron microscopy. Alterations in the endothelium, blebbing and microvilli-like projections from the luminal surface were consistently seen. In addition, the majority of cases had micro-thrombi in the low shear areas near the intercostal branches. In vitro, aortic strips from these rats showed a reduction in PGI_2 release and the platelets from these rats aggregated more readily than controls. Similarly, Lubawy, (1983) found that aortic rings from rats exposed

to cigarette smoke daily 5 times a week for 10 weeks synthesised less PGI_2 from [^{14}C] AA than sham-smoked rats. Also, the platelets from the smoke exposed rats synthesised more TXB2 from [^{14}C] AA than room control rats but not sham-smoke rats.

The responses to exogenously administered PGI_2, i.e. a fall in systolic and diastolic blood pressure and tachycardia, were significantly reduced in rats exposed to smoke 1 and 24 hours (but not 48 hours) previously. The responses to $PGF_{2\alpha}$, PGE_2 and AA were not significantly affected by prior exposure to smoke (Boura et al.,1981). There is also some evidence from human studies that smoking may affect vascular PGI_2 production. Diminished PGI_2 formation was demonstrated in umbilical arteries of babies born to mothers who smoke (Dadak et al., 1981). Busacca et al., (1982) measured PGI_2 release from rings of umbilical artery and found that, although PGI_2 release was lower by rings of smokers than non-smokers, this difference was not significant; but, cultured umbilical artery endothelial cells from smokers stimulated with AA produced significantly less PGI_2 than non-smokers. Interestingly, the success rate in establishing cultures of endothelial cells from smokers (particularly those who smoked more than 15/day) was much lower than that of non-smokers. Urinary PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) was measured in 12 chronic smokers and 12 non-smokers after inhalation of smoke from nicotine and nicotine-free cigarettes (Nadler et al., 1983). Nicotine-free cigarettes had no effect on urinary PGI_2

levels in smokers or non-smokers but the inhalation of nicotine-smoke in chronic smokers produced a highly significant reduction in urinary PGI_2 levels. In contrast, nicotine-smoke had no effect on urinary PGI_2 in non-smokers. In the present study, the effect of cigarette smoking on PGI_2, $PGF_{2\alpha}$ and PGE_2 production by homogenates of aorta and saphenous vein has been examined.

6.2.1.2 Results

The results for male and female patients were treated separately because of the differences observed in PG production between the two groups, but were not divided according to age. Although there were some differences in PG production between the female age groups it was felt that because of the small number of female subjects it might be more informative to divide the whole group into smokers and non-smokers. The patients were subdivided further into non-smokers i.e. those who had never smoked, ex-smokers; those who used to smoke but had stopped some time prior to the operation and smokers; those who were currently smoking. Tables 6.2.1 and 6.2.2 show the mean (+/-s.e.m.) production of PGs by homogenates of aorta and saphenous vein of male patients.

Table 6.2.1

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) from male non-smokers, ex-smokers, smokers and ex-smokers and smokers combined.

		Prostaglandin production (ng/mg wet wt.)			
		Non-Smokers	Ex-smokers	Smokers	Ex-smokers + smokers
n		(33)	(31)	(24)	(55)
6-Keto-$PGF_{1\alpha}$	A	14.7+/-1.7	10.6+/-1.4	12.3+/-1.6	11.3+/-1.0
	V	17.4+/-1.3	13.7+/-1.2*	13.8+/-1.8	13.8+/-1.0*
$PGF_{2\alpha}$	A	14.4+/-1.6	12.4+/-1.6	17.2+/-2.4	14.1+/-1.4
	V	8.6+/-1.0	5.7+/-0.6*	7.1+/-1.1	6.4+/-0.6*
PGE_2	A	14.2+/-1.8	12.0+/-1.7	11.7+/-1.9	12.1+/-1.2
	V	5.0+/-0.5	4.0+/-0.4	3.5+/-0.5*	3.7+/-0.3*

* Significantly ($P<0.05$) different from value for non-smokers using analysis of variance.

Table 6.2.2

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) from female non-smokers, ex-smokers, smokers and ex-smokers and smokers combined.

		PG production (ng/mg wet wt.)			
		Non-smokers	Ex-smokers	Smokers	Ex-smokers + smokers
n		(10)	(19)	(10)	(29)
6-Keto-$PGF_{1\alpha}$	A	9.3+/-0.9	12.0+/-1.7	10.7+/-2.5	11.5+/-1.4
	V	16.6+/-2.9	15.4+/-2.1	12.4+/-2.6	14.3+/-1.6
$PGF_{2\alpha}$	A	20.3+/-4.2	16.5+/-3.0	13.5+/-3.7	15.5+/-2.3
	V	8.4+/-0.8	11.0+/-1.7	8.9+/-1.5	10.3+/-1.2
PGE_2	A	13.7+/-2.5	12.0+/-2.1	10.9+/-1.5	11.6+/-1.4
	V	4.2+/-0.6	4.2+/-0.5	6.4+/-2.0	5.0+/-0.8

6.2.1.3 Conclusions

It can be seen from Table 6.2.1 that the production of 6-keto-$PGF_{1\alpha}$ is significantly greater ($P<0.05$) by homogenates of saphenous vein from male non-smokers compared to ex-smokers. Although 6-keto-$PGF_{1\alpha}$ production

was lower in veins from male smokers than non-smokers this was not significant. But, 6-keto-$PGF_{1\alpha}$ production by veins was greater by non-smokers when smokers and ex-smokers were combined ($P<0.05$). $PGF_{2\alpha}$ production was significantly lower ($P<0.05$) by homogenates of vein from male ex-smokers, or ex-smokers and smokers combined than non-smokers. PGE_2 production was significantly lower ($P<0.05$) by homogenates of vein from male smokers and ex-smokers and smokers combined compared to non-smokers. The production of all 3 PGs tended to be lower by homogenates of aorta from male smokers or ex-smokers than non-smokers but this was not significant. There were no significant diferences in the production of the 3 PGs by homgenates of aorta and vein from female non-smokers compared to smokers, ex-smokers, or smokers and ex-smokers combined (Table 6.2.2).

Section 6.2.2

The effects of cigarette smoking on PG release from saphenous vein of male and female subjects.

6.2.2.1 Introduction

The experiments described in Section 6.2.1 measured PG production by homogenates of vascular tissue. The experiments described in this section have measured PG output from the saphenous vein in relation to cigarette smoking.

6.2.2.2 Results

Male and female subjects were divided into non-smokers, ex-smokers, smokers and ex-smokers and smokers combined as in the previous sub-section. Tables 6.2.3 and 6.2.4 show the mean (+/-s.e.m.) release of PGs from rings of saphenous vein from male and female subjects respectively.

Table 6.2.3

Mean (+/-s.e.m.) release of prostaglandins (PGs) from rings of saphenous vein from male subjects.

	Prostaglandin output (ng/mg wet wt.)			
	Non-smokers	Ex-smokers	Smokers	Ex-smokers+ smokers
n	(22)	(16)	(10)	(26)
6-Keto-$PGF_{1\alpha}$	5.1+/-0.9	3.2+/-0.6	4.6+/-1.0	3.7+/-0.5
$PGF_{2\alpha}$	2.9+/-0.4	1.6+/-0.2*	2.6+/-0.4	2.0+/-0.2
PGE_2	2.1+/-0.3	1.7+/-0.2	2.4+/-0.6	2.0+/-0.3

* Significantly ($P<0.05$) different from non-smokers using analysis of variance.

Table 6.2.4

Mean (+/-s.e.m.) release of prostaglandins (PGs) from rings of saphenous vein from female subjects.

	Prostaglandin output (ng/mg wet wt.)			
	Non-smokers	Ex-smokers	Smokers	Ex-smokers+ smokers
n	(5)	(8)	(3)	(11)
6-Keto-$PGF_{1\alpha}$	5.3+/-0.5	4.9+/-1.1	4.2+/-0.2	4.7+/-0.8
$PGF_{2\alpha}$	4.1+/-1.6	2.5+/-0.4	1.3+/-0.3	2.2+/-0.3
PGE_2	2.2+/-0.2	1.6+/-0.3	1.5+/-0.2	1.6+/-0.2

6.2.2.3 Conclusions

The release of 6-keto-$PGF_{1\alpha}$ from saphenous vein rings of males tended to be less in smokers and ex-smokers than non-smokers but this was not significant (Table 6.2.3). PGE_2 release was not significantly different in smokers or ex-smokers compared to non-smokers but the release of $PGF_{2\alpha}$ was significantly ($P<0.05$) less from the vein of ex-smokers, but not smokers, compared to non-smokers. The saphenous vein from female smokers or ex-smokers tended to release lower amounts of all 3 PGs compared to non-smokers but because of the small number of subjects and the variation in the response, the difference was not significant (Table 6.2.4).

Section 6.3

The effects of β-blockers on PG production by aorta and saphenous vein of male and female subjects.

6.3.1 Introduction

Several early studies showed that β-adrenergic blocking agents depressed platelet function and inhibited platelet aggregation (Bygdeman and Johnson, 1969; Bucher and Stucki, 1969; and Frishman et al., 1974). More recent *in vivo* studies using inhibitors of PG synthesis have suggested that β-blockers may also stimulate the release of vasodilator PGs from the vasculature in rabbits (Durao and Rico, 1977) and in humans (Durao, Prata and Goncalves, 1977 and Watkins, Abbott, Hensby, Webster and Dollery, 1980). Consequently, the effect of treatment with β-blockers on PG production by homogenates of aorta and saphenous vein was investigated.

It was not possible to draw any valid conclusions on the effects of β-blockers on PG release from rings of saphenous vein as very few of this group of patients were not taking a β-adrenergic blocking agent. Similarly, with regard to subsequent sections, there were insufficient numbers of patients not taking nitrates and Ca^{2+} antagonists, and too few people taking a thiazide or loop diuretic to draw significant conclusions about the effects of these drugs on PG release from rings of saphenous vein.

6.3.2 Results

The results for males and females were considered separately because of the differences observed in PG production between the two groups. Patients were divided into those who were taking a B-blocker and those who were not receiving this form of treatment. Tables 6.3.1 and 6.3.2 show the mean (+/-s.e.m.) production of PGs by homogenates of aorta and saphenous vein of male and female patients, respectively.

Table 6.3.1

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of male subjects taking a β-blocker.

		Prostaglandin production (ng/mg wet wt.)	
		β-blocker	No β-blocker
n		(54)	(34)
6-Keto-$PGF_{1\alpha}$	A	12.3 +/- 1.1	12.5 +/- 1.4
	V	14.5 +/- 1.3	17.9 +/- 1.4
$PGF_{2\alpha}$	A	14.2 +/- 1.3	15.2 +/- 1.9
	V	7.1 +/- 0.7	7.6 +/- 0.9
PGE_2	A	12.6 +/- 1.2	13.2 +/- 1.8
	V	4.8 +/- 0.8	4.6 +/- 0.5

Table 6.3.2

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of female subjects taking a β-blocker.

		Prostaglandin production (ng/mg wet wt.)	
		β-blocker	No β-blocker
n		(26)	(13)
6-Keto-$PGF_{1\alpha}$	A	13.0 +/- 1.4 *	6.8 +/- 0.5 #
	V	15.2 +/- 1.8	13.6 +/- 2.2
$PGF_{2\alpha}$	A	17.4 +/- 2.7	15.2 +/- 3.0
	V	10.4 +/- 1.4 #	8.4 +/- 1.1
PGE_2	A	11.9 +/- 1.6	12.8 +/- 1.9
	V	5.3 +/- 1.9	3.7 +/- 0.5

* Significantly different ($P<0.01$) from value for females not taking a B-blocker using Student's t-test.

Significantly ($P<0.05$) different from corresponding male value using Student's t-test.

6.3.3 Conclusions

It can be seen from Table 6.3.1 that the use of a β-blocking agent by male patients does not affect the *in vitro* production of PGs by homogenates of aorta and saphenous vein. There was no significant difference in the production of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ or PGE_2 by aorta or vein of males taking a β-blocker compared to those who were not taking these agents. In female patients however, the production of 6-keto-$PGF_{1\alpha}$ was significantly greater ($P<0.05$) by homogenates of aorta from patients who were taking a β-blocker compared to those who were not (Table 6.3.2). The production of 6-keto-$PGF_{1\alpha}$ was not significantly different by the saphenous vein of the two groups. $PGF_{2\alpha}$ and PGE_2 production were not significantly different by either aorta or vein of females taking a β-blocker than females who were not taking these agents. Comparing male and female patients, homogenates of aorta from females not taking a β-blocker produced significantly less ($P<0.05$) 6-keto-$PGF_{1\alpha}$ than aorta from males not taking a β-blocker. In addition, homogenates of vein from females taking a β-blocker produced significantly greater ($P<0.05$) amounts of $PGF_{2\alpha}$ than the vein from males taking a β-blocker.

Section 6.4

The effects of nitrates on PG production by aorta and saphenous vein of male and female subjects.

6.4.1 Introduction

Nitrates, including isosorbide mononitrate, isosorbide dinitrate and glyceryl trinitrate (GTN) are agents used in angina pectoris and their efficacy and rapidity of action are well-established (Hillis and Braunwald, 1977).However, the mechanism of action of nitrates is poorly understood. Evidence is accumulating that at least part of the vasodilatory action of GTN is mediated through the release of PGI_2. Levin et al. (1981), showed that GTN stimulates PGI_2 release by isolated human umbilical endothelial cells in culture and Shroer et al. (1981) showed that GTN significantly increased the basal and AA-stimulated release of PGI_2 from rings of bovine coronary artery. This increase in vessel PGI_2 release inhibited TXA_2 formation in human platelets as seen when aliquot portions of the vessel incubates were added to platelets prior to thrombin. In vivo, however, although NTG decreased peripheral vascular resistance in anaesthetised dogs, blood flow did not change significantly and no significant changes in plasma 6-keto-$PGF_{1\alpha}$, PGE_1 and $PGF_{2\alpha}$ were found (Kai et al., 1982). Sakai et al. (1984), using open-chest dogs, found that intra-coronary or intra-venous NTG increased coronary blood flow but this effect was not significantly

modified by indomethacin. Although actual PG levels were not measured in the latter study it does seem that in the dog the effects of NTG are not mediated through the PG system. Nevertheless, in the present study it was thought relevant to examine the effect of treatment with nitrates on PG production by homogenates of human aorta and saphenous vein.

6.4.2 Results

The results for males and females were considered separately because of the differences observed in PG production between the two groups. The patients were separated further into those who were taking a nitrate (either GTN, isosorbide mononitrate or isosorbide dinitrate) and those who were not taking these agents. Tables 6.4.1 and 6.4.2 show the mean (+/-s.e.m.) production of PGs by homogenates of aorta and saphenous vein of male and female patients.

Table 6.4.1

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of male subjects taking a nitrate.

		Prostaglandin production (ng/mg wet wt.)	
		Nitrate	No Nitrate
n		(65)	(23)
6-Keto-$PGF_{1\alpha}$	A	12.4 +/- 1.0	13.8 +/- 1.7
	V	15.5 +/- 1.1	15.9 +/- 1.8
$PGF_{2\alpha}$	A	14.4 +/- 1.1	14.4 +/- 2.8
	V	7.2 +/- 0.6	7.4 +/- 1.2
PGE_2	A	13.7 +/- 1.2	11.0 +/- 2.2
	V	4.4 +/- 0.3	3.9 +/- 0.4

Table 6.4.2

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of female subjects taking a nitrate.

		Prostaglandin production (ng/mg wet wt.)	
		Nitrate	No nitrate
n		(30)	(19)
6-Keto-$PGF_{1\alpha}$	A	10.8 +/- 1.1	12.4 +/- 3.1
	V	16.8 +/- 1.5 *	9.0 +/- 2.6
$PGF_{2\alpha}$	A	15.6 +/- 1.9	22.3 +/- 6.4
	V	9.4 +/- 1.1	10.6 +/- 2.0
PGE_2	A	13.5 +/- 1.4	8.2 +/- 2.0
	V	5.3 +/- 0.7	3.0 +/- 0.6

* Significantly ($P<0.05$) different from value for females not taking a nitrate using Student's t-test.

6.4.3 Conclusions

It can be seen from Table 6.4.1 that taking a nitrate for the relief of angina does not affect the *in vitro* production of PGs by homogenates of aorta and saphenous

vein from male patients. The saphenous vein from female patients taking nitrates however, produced significantly ($P<0.05$) greater amounts of 6-keto-$PGF_{1\alpha}$ than the vein from females not taking these drugs (Table 6.4.2). There was no significant difference in the production of 6-keto-$PGF_{1\alpha}$ by the aorta of females taking nitrates compared to females who were not taking them. There was no significant difference in the production of $PGF_{2\alpha}$ and PGE_2 by either aorta or vein of females taking nitrates. PG production did not differ significantly by aorta or vein of males compared to females.

Section 6.5

The effects of thiazide diuretics on PG production by aorta and saphenous vein of male and female subjects.

6.5.1 Introduction

The thiazide diuretics, commonly used in anti-hypertensive therapy, may have effects on PG formation. Indomethacin blunted the fall in blood pressure after administration of thiazide diuretics in humans (Watkins et al., 1980) and in a complimentary study, increased levels of 6-keto-$PGF_{1\alpha}$ were found in the plasma of hypertensive patients given bendrofluazide (Webster, Dollery and Hensby, 1980). The effect of thiazide diuretic treatment on PG production by homogenates of human aorta and saphenous vein have therefore been examined.

6.5.2 Results

The results for male and female patients were treated separately because of the differences observed in PG production between these two groups. Patients were divided into those who were taking a thiazide diuretic and those who were not receiving this form of treatment. Tables 6.5.1 and 6.5.2 show the mean (+/-s.e.m.) production of PGs by homogenates of aorta and saphenous vein of male and female patients.

Table 6.5.1

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of male subjects taking a thiazide diuretic.

		Prostaglandin production (ng/mg wet wt.)	
		Thiazide	No Thiazide
n		(12)	(75)
6-Keto-$PGF_{1\alpha}$	A	10.3 +/- 2.0	12.7 +/- 0.9
	V	15.5 +/- 2.1	16.1 +/- 1.1
$PGF_{2\alpha}$	A	13.0 +/- 3.4	14.5 +/- 1.2
	V	9.6 +/- 2.4	6.8 +/- 0.5
PGE_2	A	12.2 +/- 3.2	13.1 +/- 1.0
	V	3.9 +/- 0.7	4.3 +/- 0.3

Table 6.5.2

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of female subjects taking a thiazide diuretic.

		Thiazide	No Thiazide
n		(11)	(28)
6-Keto-$PGF_{1\alpha}$	A	12.5 +/- 2.2	10.4 +/- 1.3
	V	19.4 +/- 2.3 *	13.3 +/- 1.7
$PGF_{2\alpha}$	A	13.7 +/- 3.2	18.4 +/- 2.6
	V	9.6 +/- 1.1	9.6 +/- 1.3
PGE_2	A	8.8 +/- 1.0	13.9 +/- 1.6
	V	6.4 +/- 1.8	4.2 +/- 0.5

* Significantly ($P<0.05$) different from value for females not taking a thiazide diuretic.

6.5.3 Conclusions

There was no significant difference in the production of any of the 3 PGs by aorta or saphenous vein of male patients taking a thiazide diuretic compared to males not taking these drugs. Similarly, thiazide diuretics did not appear to have a significant effect on the *in vitro* production of $PGF_{2\alpha}$ and PGE_2 by aorta and vein of female patients. Although 6-keto-$PGF_{1\alpha}$ production by aorta did not differ between the two groups, 6-keto-$PGF_{1\alpha}$ production was significantly greater by saphenous vein of females taking a thiazide diuretic.

Section 6.6

The effects of loop diuretics on PG production by aorta and saphenous vein of male and female subjects.

6.6.1 Introduction

Frusemide is a well-known loop diuretic which has a pronounced anti-hypertensive action. Its mode of action appears to be related to the PG system as cyclooxygenase inhibitors partially block the natriuretic response and the effects on renal blood flow (Bailie, Crosslan and Hook, 1976; Williamson, Bourland and Marchand, 1974). It has been suggested that frusemide may also have extra-renal effects on PG formation. Weithman (1982) found that aortic rings from rats treated with therapeutic concentrations of frusemide showed a

significantly higher release of a PGI_2-like substance which was supressed by pre-treatment of the rats with indomethacin or aspirin. However, frusemide did not stimulate PGI_2-like release from rat aortas *in vitro* suggesting that it acts *in vivo* via secondary factors present in plasma. Nevertheless, it was considered relevant to examine the effects of loop diuretics on PG production by homogenates of human aorta and saphenous vein.

6.6.2 Results

The results for males and females were considered separately. Patients were divided into those who were taking a loop diuretic (frusemide or bumetanide) and those who were not receiving this form of treatment. Tables 6.6.1 and 6.6.2 show the mean (+/-s.e.m.) production of PGs by homogenates of aorta and vein of male and female patients.

Table 6.6.1

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of male subjects taking a loop diuretic.

		Prostaglandin production (ng/mg wet wt.)	
		Loop diuretic	No loop diuretic
n		(14)	(74)
6-Keto-$PGF_{1\alpha}$	A	11.8 +/- 2.1	12.3 +/- 0.9
	V	17.7 +/- 3.2	15.4 +/- 1.0
$PGF_{2\alpha}$	A	14.7 +/- 2.9	14.0 +/- 1.2
	V	5.4 +/- 0.5	7.8 +/- 0.6
PGE_2	A	10.5 +/- 2.2	13.6 +/- 1.1
	V	3.7 +/- 0.6	4.3 +/- 0.3

Table 6.6.2

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of female subjects taking a loop diuretic.

		Prostaglandin production (ng/mg wet wt.)	
		Loop diuretic	No loop diuretic
n		(9)	(30)
6-Keto-$PGF_{1\alpha}$	A	8.0 +/- 1.1	12.1 +/- 1.3
	V	13.8 +/- 2.7	15.5 +/- 1.7
$PGF_{2\alpha}$	A	17.0 +/- 4.7	17.1 +/- 2.3
	V	7.7 +/- 1.2	10.2 +/- 1.2
PGE_2	A	11.6 +/- 1.9	12.7 +/- 1.5
	V	5.0 +/- 0.9	4.8 +/- 0.8

6.6.3 Conclusions

It can be seen from Tables 6.6.1 and 6.6.2 that the use of a loop diuretic does not affect the *in vitro* production of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ or PGE_2 by aorta or saphenous vein of male and female patients.

Section 6.7

The effects of calcium antagonists on PG production by aorta and saphenous vein of male and female subjects.

6.7.1 Introduction

Calcium antagonists such as nifedipine and verapamil have been widely used for angina pectoris although their mechanism of action has not been fully elucidated. One study by Srivistava and Awasthi (1983) has shown that although nifedipine and verapamil actually reduced PGI_2 release from rings of rat aorta in response to stimulation with AA, they increased the formation of PGI_2 by lung homogenates incubated with AA. In anaesthetised dogs nifedipine produced a decrease in peripheral vascular resistance but there was no significant change in plasma 6-keto-$PGF_{1\alpha}$, PGE_1 or $PGF_{2\alpha}$. In contrast, verapamil produced a decrease in peripheral vascular resistance but significantly increased plasma levels of 6-keto-$PGF_{1\alpha}$, PGE_1 and $PGF_{2\alpha}$. Indomethacin reduced the effects of verapamil on vascular resistance and plasma PG concentration (Kai et al., 1982). Consequently, the effects of calcium antagonists on PG production by homogenates of human aorta and saphenous vein have been examined.

6.7.2 Results

The mean (+/-s.e.m.) production of PGs by homogenates of aorta and saphenous vein of male and female subjects taking nifedipine or verapamil is shown in Tables 6.7.1 and 6.7.2 respectively.

Table 6.7.1

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of male subjects taking nifedipine (n=52) or verapamil (n= 2), (Ca^{2+} antag.).

		Prostaglandin production (ng/mg wet wt.)	
		Ca^{2+} antag.	No Ca^{2+} antag.
n		(54)	(34)
6-Keto-$PGF_{1\alpha}$	A	10.8 +/- 1.0 *	14.5 +/- 1.5
	V	14.4 +/- 1.2	17.9 +/- 1.6
$PGF_{2\alpha}$	A	13.7 +/- 1.3	15.4 +/- 1.9
	V	6.4 +/- 0.5	8.5 +/- 1.1
PGE_2	A	12.2 +/- 1.2	14.2 +/- 1.8
	V	4.7 +/- 0.4	4.5 +/- 0.5

* Significantly different ($P<0.05$) from male subjects not taking nifedipine.

Table 6.7.2

Mean (+/-s.e.m.) production of prostaglandins (PGs) by homogenates of aorta (A) and saphenous vein (V) of female subjects taking nifedipine (n=17) or verapamil (n= 3), (Ca^{2+} antag.).

		Prostaglandin production (ng/mg wet wt.)	
		Ca^{2+} antag.	No Ca^{2+} antag.
n		(20)	(19)
6-Keto-$PGF_{1\alpha}$	A	11.2 +/- 1.5	10.6 +/- 1.6
	V	15.8 +/- 2.0	14.0 +/- 2.1
$PGF_{2\alpha}$	A	17.0 +/- 3.1	17.0 +/- 2.8
	V	9.4 +/- 1.7	9.5 +/- 0.9
PGE_2	A	13.2 +/- 2.2	11.8 +/- 1.2
	V	4.4 +/- 0.6	5.2 +/- 1.1

6.7.3 Conclusions

The aorta from male patients taking a Ca^{2+} antag produced significantly lesser ($P<0.05$) amounts of 6-keto-$PGF_{1\alpha}$ than the aorta from males not taking this drug (Table 6.7.1). There were no significant differences in the production of 6-keto-$PGF_{1\alpha}$ by vein, or by $PGF_{2\alpha}$ and PGE_2 by either aorta or vein between the two groups. The Ca^{2+} antag. did not appear to have any effect on the production of the 3 PGs by either aorta or vein of female patients (Table 6.7.2).

6.8 Discussion

The results of the present study do not show any significant differences in the production of 6-keto-$PGF_{1\alpha}$ by homogenates of aorta or vein of male subjects with age. Also, there was no difference in 6-keto-$PGF_{1\alpha}$ output from rings of saphenous vein with age. There was a significant decrease in the release of PGE_2 from rings of saphenous vein of males aged 61-70 compared to males of 41-50. 6-Keto-$PGF_{1\alpha}$ production was significantly lower by homogenates of saphenous vein of females aged 41-50 and 61-70 years than males in the same age groups but females aged 41-50 and 61-70 produced greater amounts of $PGF_{2\alpha}$ from homogenised veins than the corresponding male groups. Similarly, rings of saphenous vein from females aged 61-70 years released significantly greater amounts of $PGF_{2\alpha}$ than males in the same age group. There were no other sex differences observed in PG production or release. Because there were only 2 males and 2 females aged 70 years or more it was not possible to comment on either age or sex differences in this small sample of elderly patients compared to the other age groups.

This study has shown that homogenates of saphenous vein from male ex-smokers or ex-smokers + smokers produce significantly less 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 compared to non-smokers. However, the basal release of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 from rings of saphenous vein, although lower in smokers or ex-smokers, did not differ significantly to non-smokers, except for a

significantly lower output of $PGF_{2\alpha}$ im male ex-smokers. Interestingly, reduced PG production by vein was not seen in female patients, although PG release from rings of saphenous vein did appear to be lower in smokers. It is not possible to say if this is a genuine difference in the response of male and female vascular tissue to cigarette smoke because the number of female subjects was rather small.

Of the patients taking a β-blocker only female subjects showed any significant differences in PG production, the aorta producing more 6-keto-$PGF_{1\alpha}$ than the aorta from females not taking a β-blocker. The use of a β-blocker had no effect on the *in vitro* production of PGs by vascular tissue of male subjects. Nitrates produced a similar effect to β-blockers with respect to PG production. Males taking nitrates did not show any significant difference in vascular PG production but the saphenous vein from females taking nitrates produced significantly greater amounts of 6-keto-$PGF_{1\alpha}$ than females who were not taking this treatment. The calcium antagonist, nifedipine and the loop diuretic, frusemide did not appear to have any marked effects on *in vitro* PG production by aorta and vein of male or female subjects. However, the production of 6-keto-$PGF_{1\alpha}$ by the aorta of males taking nifedipine was significantly less than that of males not taking nifedipine. Other drugs known to affect PG production such as NSAIDs and dipyridamole have not been discussed here because the number of patients taking these drugs (9 and 4 respectively) was too small

for valid comparisons to be made. However, as there did not appear to be a marked effect of either of these two drugs on PG production by homogenates of aorta and saphenous vein, these patients were included in the analyses.

SECTION 7.0

Discussion

7.1.1 Profile of PG production by several blood vessels from male and female rats.

Since the discovery of PGI_2, and in particular the ability of blood vessels to produce this potent vasodilatory and antiaggregatory PG, it has been assumed that PGI_2 is the major and most important product of arachidonic acid metabolism in all blood vessels. This has led to the formulation of an hypothesis of a TXA_2/PGI_2 balance controlling blood vessel homeostasis. However, this view may be an over-simplification of the involvement of arachidonic acid metabolites in the control of blood vessel tone and platelet aggregation.

The initial experiments described in this thesis have established that, contrary to the accepted view, PGI_2 is not the sole product of AA in blood vessels and it has been demonstrated that aorta, vena cava, mesenteric artery and femoral vein of male and female rats all produce substantial amounts of PGE_2 and $PGF_{2\alpha}$ in addition to PGI_2 (measured as 6-keto-$PGF_{1\alpha}$). This was true for homogenates of all 4 blood vessels, and also for the perfused aorta and the perfused mesenteric arterial bed. Indeed, homogenates of the vena cava, mesenteric artery and femoral artery from male rats produced similar amounts of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2, and it was only in the aorta that 6-keto-$PGF_{1\alpha}$ production was markedly

greater than that of $PGF_{2\alpha}$ and PGE_2. In the female, the vena cava produced similar amounts of the 3 PGs, the mesenteric and femoral arteries produced mainly 6-keto-$PGF_{1\alpha}$ with less $PGF_{2\alpha}$ and PGE_2, and, in contrast to the situation in the male the aorta produced similar amounts of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ with less PGE_2. Also, using the experimental procedures of perfusion of an intact vessel and perfusion of an entire resistance bed, 6-keto-$PGF_{1\alpha}$ was the PG released in greatest quantities by the isolated, perfused aorta and the isolated, perfused mesenteric vascular bed of both male and female rats. These two preparations probably give a better representation of the profile of the basal output of PGs from a blood vessel than the use of rings or strips of aorta which will reflect the release of PGs from not only the endothelium but also the cut ends of the smooth muscle layer. As the procedure of cutting the blood vessel into rings will in itself cause PG formation, via stimulation of PLA_2, this type of preparation may not give an accurate representation of the basal, unstimulated release of PGs from vascular tissue.

Many studies in different species have shown that PGI_2 is the principal PG synthesised in large conduit type blood vessels such as the aorta. In contrast, microvessels and microvascular endothelial cells, such as from rabbit myocardium or bovine cerebral cortex exhibit a very different PG profile with PGE_2 being the major PG produced both under stimulated and basal conditions (Gerritsen and Cheli, 1983; Gerritsen and Printz, 1981).

Furthermore, PGI_2 is not the only PG involved in the modulation of contractility of blood vessels. In the rabbit, exogenous PGI_2 inhibited contractions induced by NA in the coeliac artery but was without effect on the aorta, extrapulmonary artery or femoral artery; PGE_2 had no effect on NA-induced contractions of the aorta, inhibited those of the pulmonary and coeliac arteries, and markedly potentiated those of the femoral artery; $PGF_{2\alpha}$ significantly enhanced contractions of the femoral artery, but the increased contractions of the other vascular preparations were not significant (Foerstermann, Herrting and Neufang, 1984). Salzman et al. (1980) demonstrated similar effects of PGs on intrapulmonary arteries with PGI_2 having no consistent effect, $PGF_{2\alpha}$ slightly contracting the tissue and PGE_2 always relaxing the tissue. In the canine mesenteric artery strip, $PGF_{2\alpha}$ and PGE_2 produced contraction of the tissue whereas PGE_1 and PGI_2 were equally potent at causing relaxation (Hatano, Kohli, Goldberg and Fried, 1981). These findings suggest that in certain blood vessels, PGs other than PGI_2 may be important modulators of vascular tone, and indicate that not all changes in vascular tone following cyclooxygenase inhibition should be interpreted as due solely to the inhibition of PGI_2 synthesis. It is important, therefore, in studies of vascular PG production that the fullest range of PGs is measured whenever possible.

7.1.2 Male and female differences in vascular PG production

The majority of studies on vascular PG production have been carried out using male animals, although it has been reported that PGI_2 output from the rat aorta, and from perfused lungs, is higher in male than in female rats (Pomerantz et al., 1980; Maggi et al., 1980; Wey et al., 1983; Bakhle and Zakrzewski, 1981). In agreement with these authors the findings of the present study also showed an increased output of 6-keto-$PGF_{1\alpha}$ from the isolated, perfused aorta of male compared to female rats. The total amount of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 synthesised by homogenates of aorta did not differ between male and female rats, therefore the higher basal output of 6-keto-$PGF_{1\alpha}$ from the aorta of male rats cannot be due to a greater concentration of PGH_2 synthetase in the aorta of males compared to females. However, there did appear to be a slight shift in PG synthesis away from 6-keto-$PGF_{1\alpha}$ towards $PGF_{2\alpha}$ in the female aorta and this may be responsible for the lower output of 6-keto-$PGF_{1\alpha}$ from the aorta of female rats. It is of interest that despite the aorta from male and female rats having a similar capacity to produce PGI_2, it appears that the male aorta, in a situation where the aorta is perfused in a pulsatile manner to more closely simulate *in vivo* conditions, is more active in releasing PGI_2 than the female aorta. 6-Keto-$PGF_{1\alpha}$ production was greater by vena cava and lower by femoral artery of male compared to female rats. The amounts of $PGF_{2\alpha}$ and PGE_2 produced by vena cava and femoral artery of males and females were not significantly different. The production of 6-keto-$PGF_{1\alpha}$,

$PGF_{2\alpha}$ and PGE_2 by the mesenteric artery did not differ between the sexes.

7.1.3 Vascular PG production at different times during the oestrous cycle.

Oestrogen or a combination of oestrogen/progestagen treatment has been reported to stimulate the output of PGI_2 from the aorta and vena cava of female rats (Roncaglioni et al., 1979; Ali and Williams, 1983; Karpati et al.,1980) and from rat aortic smooth muscle cells *in vitro* (Chang et al., 1980). The reasons for the increased output of PGI_2 from the aorta of male rats and the possible role of oestrogen in vascular arachidonic acid metabolism have not been elucidated. Studies presented in this thesis aimed to establish whether the levels of oestrogen and progesterone produced during the female rats' natural cycle could affect aortic PG synthesis. The two periods of the oestrous cycle investigated in this study were 10:00 h on the day of pro-oestrus, i.e. 4h after a peak of oestradiol and when progesterone levels are still low, and 02:00 h on the day of oestrus, i.e. 4 h after a peak of progesterone and when plasma oestradiol levels are declining. These times were chosen to ensure that if there were differing effects of oestrogen and progesterone on PG synthesis, then sufficient time had elapsed for there to be fresh protein synthesis.

There was no significant difference in PG production by homogenates of the aorta or the vena cava at these two

times during the oestrous cycle. Ali and Williams, (1983) found that PGI_2 release from rings of aorta was lowest at oestrus and significantly greater at pro-oestrus, met-oestrus and di-oestrus and showing a similar fluctuation to the amounts of PGI_2 released by the myometrium. Isolated lungs from rats at different stages of the oestrous cycle when perfused with [^{14}C] AA were found to produce more 6-keto-$PGF_{1\alpha}$ at pro-oestrus than at any other stage of the cycle (Bakhle and Zakrzewski, 1981). The majority of work on the effects of the oestrous cycle on PG production has understandably been carried out using uterine tissue, and it is interesting that the observations made using isolated lungs are in agreement with several workers reporting that the PG-synthesising ability of the uterus is greatest at pro-oestrus (Thaler-Dao, Saintot, Ramonatxo, Chavis and Crastes de Paulet, 1982) and in conflict with the findings of other workers that the capacity for uterine PG synthesis is greatest at oestrus (Poyser and Scott, 1980; Ham, Cirillo, Zanetti, and Kuehl, 1975). However, measurements have largely been made only once or twice daily during the cycle and the study of Brown and Poyser (1985) has shown the importance of measuring more frequently than this (every 4 hours) to determine the times of maximum PG production in the uterus. PG production by homogenates of the uterus was maximal at 02:00 h on the day of oestrus. A similar protocol of 4-hourly measurements of PG-synthesising capacity during the oestrous cycle, adopted for the study of blood vessel tissue, would possibly be required to reveal an effect,

if any, of endogenous oestrogen and/or progesterone on vascular PG production.

7.1.4 Effects of oestrogen and progesterone treatment on vascular PG production.

Since there were no significant differences in vascular PG production at the two times of the cycle studied here, the effects of short-term treatment with oestrogen and progesterone in an acute ovariectomy model were investigated. There were no significant differences in the production of any one PG by either the aorta or vena cava of oestrogen- and progesterone- treated rats compared to control, ovariectomised rats. However the procedure of ovariectomy in itself appears to have had a significant effect on vascular PG production. Comparing intact Day 1 or Day 4 rats to ovariectomised control or treated rats, there was a significant increase in 6-keto-$PGF_{1\alpha}$ production by aorta of ovariectomised rats, and a significant increase in PGE_2 production by vena cava from ovariectomised compared to intact rats. These findings are in agreement with those of Wey et al., (1983) who found that neonatal ovariectomy increased the capacity of the aorta for 6-keto-$PGF_{1\alpha}$ synthesis, and Pomerantz et al., (1981) who found that ovariectomy of rats at 3 weeks of age increased aortic synthesis of 6-keto-$PGF_{1\alpha}$ as an adult. Deprivation of ovarian steroid hormones may therefore have an inhibitory effect on vascular PGs but the effect is a long-term, as opposed to a short-term, effect on PG production, as short-term

treatment with steroids did not reverse the effects of ovariectomy.

7.2 Vascular PG production in hypertensive rats.

Homogenates of aorta from normotensive and hypertensive male rats synthesised mainly 6-keto-$PGF_{1\alpha}$ with less PGE_2 and $PGF_{2\alpha}$, whereas homogenates of vena cava synthesised 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ in similar quantities, with less PGE_2. There was no difference in 6-keto-$PGF_{1\alpha}$ or PGE_2 synthesis in homogenates of aorta or vena cava in normotensive compared to GH rats. However, $PGF_{2\alpha}$ production was significantly greater by homogenates of aorta, and tended to be greater in homogenates of vena cava from normotensive than GH male rats. The synthetic capacity of the adventitia for PG production was low in both GH and normotensive rats and the 3 PGs were produced in similar amounts, suggesting that PG production by the adventitia is of little importance in the regulation of blood vessel tone. The smooth muscle layer however, was almost as active as the endothelium in its ability to produce PGs when the results were expressed as production by the total layer. The total amounts of 6-keto-$PGF_{1\alpha}$ synthesised by the separate layers were in the order of smooth muscle > adventitia > endothelial cells except in male hypertensive rats where 6-keto-$PGF_{1\alpha}$ synthesis by the adventitia and endothelial cells was similar. The total amounts of $PGF_{2\alpha}$ and PGE_2 synthesised were also in the order of smooth muscle > adventitia > endothelial cells. However, the endothelium constitutes approximately

2% of the total aortic tissue meaning that the synthetic capacity for 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 is 3-30 fold higher in the endothelial cells than in the adventitia or smooth muscle of all groups of rats. This is in agreement with the findings of Moncada et al. (1977), using separated layers of rabbit aorta, where PGI_2 generation was highest at the intimal surface and progressively decreased towards the adventitial surface. Notwithstanding, the production of PGs may be an important function of smooth muscle *in vivo*.

The observation of greatest interest was the finding that the smooth muscle layer of hypertensive males produced significantly more $PGF_{2\alpha}$ than the smooth muscle layer from normotensives. This finding of the difference in the metabolism of AA in the aorta of hypertensive rats has not been reported before. There was a concomitant decrease in the production of 6-keto-$PGF_{1\alpha}$ in the smooth muscle layer from GH male rats. The endothelial cell layer from GH rats also produced significantly greater amounts of $PGF_{2\alpha}$ then normotensives but as PGE_2 production was at the same time decreased, it is not possible to discount that the increased amount of $PGF_{2\alpha}$ measured was due to increased activity of 9-PGE_2-ketoreductase in GH rats, thus increasing conversion of PGE_2 to $PGF_{2\alpha}$. There was no difference in the output of 6-keto-$PGF_{1\alpha}$ or PGE_2 from the isolated aorta between the two groups of male rats. However, the release of $PGF_{2\alpha}$ from the aorta of GH rats was markedly increased (3-fold) compared to normotensives and this was

possibly a reflection of the increased synthetic capacity of the aortic smooth muscle and endothelium from hypertensives to produce $PGF_{2\alpha}$.

The capacity of the smooth muscle and endothelial cell layers for 6-keto-$PGF_{1\alpha}$ and PGE_2 synthesis was significantly lower in hypertensive than in normotensive female rats, yet the basal release of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 from the perfused aorta were all higher in the hypertensive state. Although GH females were similar to GH males in releasing greater amounts of $PGF_{2\alpha}$ from the aorta it is possible that the concomitant increase in PGI_2 and PGE_2 output compensates for the increase in $PGF_{2\alpha}$, assuming they can all affect the smooth muscle layer.

Similar to the observations made using homogenates of aorta, 6-keto-$PGF_{1\alpha}$ release from the intact aorta was not different between normotensive and GH rats. This is not in agreement with the findings of Okuma et al., (1980) and Pace-Asciak et al. (1978) using aortic rings from SHR, or of Botha et al., (1980) using aortic strips from the GH strain of hypertensive rats. However, the spontaneous release of PGI_2 from aortic rings or strips is probably due to the metabolism of arachidonic acid released by PLA_2 from the damaged cells, and therefore may reflect the ability of the damaged tissue to synthesise PGI_2, rather than the true basal rate of synthesis. If the increased output of PGI_2 in the

hypertensive state were a compensatory measure to alleviate the increased peripheral resistance characteristic of hypertension, as suggested by some authors, it seems inappropriate that such an obviously ineffective measure would be adopted by the body! Conversely, the increased PGI_2 output observed in the vasculature of hypertensive rats could be secondary to the mechanical effect of a raised blood pressure (as discussed in the General Introduction).

However, unlike the majority of work on blood vessels from hypertensive rats, an increase in either the production or basal release of PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) was not observed from the vessels of GH rats. However, the most marked difference between the two groups of male rats was the increased production of $PGF_{2\alpha}$ by:- (i) homogenates of whole aorta, (ii) homogenates of smooth muscle, (iii) the endothelial cell suspensions, and the increased basal output of $PGF_{2\alpha}$ from the perfused aorta and mesenteric arterial bed of hypertensives compared to normotensives. The increased production of $PGF_{2\alpha}$ by homogenates of aorta from male hypertensive rats was not due to its decreased metabolism, conversion of $PGF_{2\alpha}$ to its 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ metabolites being similar in normotensive and GH rats. Female hypertensive rats however, showed a decreased production of both 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ by the smooth muscle and endothelial cell layers but an increased production of PGE_2 compared to normotensives. In contrast to the

synthetic capacity of homogenates, the basal output of $PGF_{2\alpha}$ was greater by the perfused aorta and mesenteric bed of hypertensive than normotensive females.

Since the discovery of PGI_2 and its being 'labelled' as the major and most important product of AA metabolism in blood vessels, $PGF_{2\alpha}$ and PGE_2 have either been ignored or deemed unimportant. Yet, both of these PG's have potent effects on blood pressure. In the rat and dog $PGF_{2\alpha}$ is pressor; intra-arterial administration of $PGF_{2\alpha}$ increases vascular resistance, reportedly through a selective venoconstrictor action (DuCharme and Weeks, 1967; DuCharme et al., 1968). In the micro-circulation of the cat (pial vessels), topical administration of $PGF_{2\alpha}$ produces significant arterial constriction (Welch et al., 1974), and in the rat, arteriolar constrictor responses to $PGF_{2\alpha}$ have been observed in the mesocaecum (Vignera et al., 1969) and cremaster muscle (Ellis and Hutchins, 1974).

The observation that $PGF_{2\alpha}$ release was greater from the mesenteric bed of hypertensive male and female rats compared to their normotensive controls is of considerable interest in relation to the potent vasoconstrictor actions of $PGF_{2\alpha}$. In the dog, $PGF_{2\alpha}$ is a potent mesenteric vasoconstrictor (Chapnick et al., 1978). It also enhances the vasoconstrictor response following nerve stimulation (Hedquist, 1976) and there are reports that AII induces the release of $PGF_{2\alpha}$ from the canine kidney (Dunn, Liard and Dray, 1978). Thus,

although there was no actual stimulation of $PGF_{2\alpha}$ release in the mesenteric bed of hypertensive rats after NA and AII, the observation that $PGF_{2\alpha}$ was always present in higher amounts in these animals may be of relevance to the greater pressor response found in these animals.

7.2.1 Effects of NA and AII on PG release from the mesenteric arterial bed.

In agreement with previous studies, NA stimulated PGI_2 output and, to a lesser extent, PGE_2 output from the mesenteric arterial bed of normotensive and hypertensive male rats (Pipili and Poyser, 1982; Desjardins-Giasson et al., 1982). The present study has shown that NA will stimulate also the release of PGI_2 and, to a lesser extent, PGE_2 from normotensive and GH female rats. The potent vasodepressor actions of PGE_2 are well documented but the effects of PGE_2 are not universally vasodepressor in different vascular beds of the rat. In the splenic vasculature and cremaster muscle, PGE_2 produces vasodilation (Malik, 1979; Messina et al., 1974) but the situation in the kidney is less clear. Inhibition of PG synthesis produced vasoconstriction, suggesting that an influence of vasodilator PGs had been reduced (Dusing et al., 1977), but direct administration of PGE_2 into the rat kidney increased vascular tone (Malik and McGiff, 1975; Gerber and Neis, 1979). In the rat mesenteric arterial bed and hind limb, PGE_2 potentiates and PGI_2 attenuates the pressor response to NA whereas, in the splenic artery, PGI_2 and PGE_2 both attenuate the effect

of NA (Kondo, Okuno, Suzuki and Saruta, 1980). In contrast, PGE_2 attenuates the pressor response of NA in the mesenteric arterial bed (Malik, Ryan and McGiff, 1976). PGE_2 is believed to act by reducing the amount of transmitter released, whereas PGI_2 does not act in this way and probably has a direct action on myogenic tone. In vivo, the systolic and diastolic blood pressure responses to NA were significantly potentiated in anaesthetised rats treated with aspirin or indomethacin. The infusion of PGI_2 counteracted this aspirin or indomethacin induced potentiation of blood pressure responses to NA (Fischetti, Carmignani, Marchetti, Ranelletti and Caprino, 1980)

In agreement with previous studies, AII increased the release of PGI_2, and to a lesser extent, PGE_2 from the mesenteric arterial bed of normotensive male rats (Dusting et al., 1981; Nolan et al., 1981; Desjardins-Giasson et al., 1982). In contrast, AII failed to increase PGI_2 or PGE_2 output from the mesenteric bed of hypertensive male rats and the pressor response to AII was higher in the mesenteric bed of hypertensive compared to normotensive male rats. The importance of this finding in relation to hypertension remains to be established. The results from the female rats make interpretation of these findings more difficult, as the higher dose of AII stimulated PGI_2 output in female hypertensive rats, and tended to stimulate PGI_2 output in female normotensive rats. The pressor response to AII (1.0 ug) did not differ

significantly between hypertensive and normotensive female rats.

Studies using cyclooxygenase inhibitors in rabbits and sheep have indicated that the pressor effect of AII is normally attenuated by PGs released in response to AII treatment (Blumberg et al., 1977; Beilby, Coghlan, Denton, Graham, Humphrey, Scoggins and Whitworth, 1981; Rave and Nasjletti, 1983). In AII-induced hypertension in the rat, systolic blood pressure was positively correlated with both plasma 6-keto-$PGF_{1\alpha}$ concentration and the amount of 6-keto-$PGF_{1\alpha}$ released from rings of aorta (Diz, Baer and Nasjletti, 1983). Therefore, the results from male hypertensive rats are suggestive of an involvement of the lack of vascular PGI_2 in response to AII being causal in the raised blood pressure characteristic of these rats. The results of this study have demonstrated that when there was a stimulation of PG release by either NA or AII, it was the concentration of 6-keto-$PGF_{1\alpha}$ in the perfusion fluid which increased whereas PGE_2 and $PGF_{2\alpha}$ did not change significantly, except for a significant stimulation of PGE_2 output in normotensive males by AII.

It remains to be established if the conversion of PGI_2 to the longer acting 6-keto-PGE_1 is important for the actions of PGI_2 *in vivo*. It would be extremely interesting to measure either the conversion of PGI_2 to 6-keto-PGE_1 by liver and platelets, or the actual levels

of 6-keto-PGE_1 present in the liver and plasma of hypertensive rats to determine whether the increased, or similar levels of PGI_2 in the hypertensive state in the face of an elevated blood pressure is due to the absence of conversion of PGI_2 to 6-keto-PGE_1.

7.2.2 Effects of diet and sodium intake on vascular PG production.

Although PGE_2 and $PGF_{2\alpha}$ have frequently not been measured in studies of vascular PG production, there is some evidence from the literature that the output of these two PGs, particularly $PGF_{2\alpha}$, is altered in humans with essential hypertension and in rat models of hypertension. The release of PGE_2 from renal medullary tissue of SHR was found to be raised during the pre-hypertensive and early hypertensive stages, but decreased later with the further development of hypertension. The release of $PGF_{2\alpha}$ from renal tissue of SHR also tended to be higher than controls, but this difference was not significant (Ozawa, Kan, Konishi, Kitayima and Matsomura, 1982). The output of $PGF_{2\alpha}$ from the kidney is higher than normal in humans with essential hypertension (Weber et al., 1979) and is raised even higher if the patients are given an acute sodium load (Neri Serneri, Castellani, Scarti, Trotta, Sciagra and Masotti, 1985). In the SHR an increased renal $PGF_{2\alpha}$ formation was found even before overt hypertension had developed (Ahnfelt-Rohne and Arrigoni-Martelli, 1978). PGE_2 output however, did not change. These observations made using the kidney from hypertensive rats and the findings made using the aorta in the present

study, support the idea that an alteration in the pathway of PG formation from PGE_2 to $PGF_{2\alpha}$ may lead to an increase in blood pressure, and that it is the ratio of these PGs that is critical for blood pressure control rather than the absolute amounts of vasodilator PGs.

Further support for this hypothesis has come from studies of dietary fatty acid manipulation. Compared with a linoleic acid-rich diet, a linoleic acid-deficient diet produced an increase in the blood pressure of normotensive salt-loaded rats. This increase in blood pressure was associated with a reduction in renal and aortic PGE_2 formation, with little change in the formation of $PGF_{2\alpha}$, thus leading to an increase in the $PGF_{2\alpha}$:PGE_2 ratio (Hoffman, Taube, Ponicke, Zehl, Beitz, Forster, Somoa, Orbetzova and Davidova, 1982). The enzyme 9-PGE_2-ketoreductase is responsible for the conversion of PGE_2 into $PGF_{2\alpha}$ and it is possible that it is the increased activity of this enzyme that is causing the shift in PG production away from PGE_2 towards $PGF_{2\alpha}$ (Ziboh et al, 1977). After salt-loading, Weber et al., (1977) and Scherer, Siess and Weber (1977) found an increased activity of the 9-PGE_2-ketoreductase in the kidney of rabbits. It appears that there is a marked stimulation of this enzyme when the two treatments, linoleic-acid deficiency and salt-loading are administered concomitantly. Interestingly, blood pressure could be reduced in linoleic acid deficient salt-loaded animals by injection of gold salts, which selectively inhibit the synthesis of $PGF_{2\alpha}$ and stimulate that of PGE_2

(Hoffman and Forster, 1981; Stone, Kather and Gipson, 1975). In a similar study, a linoleic acid-deficient diet was again found to produce an increase in the blood pressure of normotensive rats and a decrease in the release of PGI_2 from isolated aorta and a secondary rise in plasma TXA_2 (measured as TXB_2) concentration (Duesing, Scherhag, Glaenzer, Budde and Kramer, 1983). Conversely, dietary deprivation of linoleic acid in SHR induced a reduction in blood pressure compared to SHR fed a high linoleic acid diet (Mogenson and Box, 1982).

A high sodium chloride diet produced a sustained and significant increase in PGI_2 formation (measured as urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$) in the Dahl salt-resistant strain of rats, which remained normotensive. Salt-loading in the Dahl salt-sensitive rats however, produced a slight decrease in PGI_2 production and a gradual increase in the blood pressure of these rats (Falardeau and Martineau, 1983). In the mesenteric arterial bed of rabbits, chronic sodium loading caused a significantly smaller release of PGI_2 following AII stimulation, and vasoconstrictor responses to AII were greater in the high sodium group (Miyamori, Yasuhara, Ikeda, Koshida, Takeda, Morise, Takimoto and Takeda, 1983).

7.2.3 Possible alteration in vascular AA metabolism in hypertension.

The vasodepressor actions of AA are enhanced in both SHR and 1K, 1C hypertensive rats (Dusting, Di Nicolantonio,

Drysdale and Doyle, 1981; Dusting, Dickens, Di Nicolantonio and Doyle, 1984). The increased efficiency of the CO pathway in these 2 models of hypertension perhaps results from a more fundamental defect in AA metabolism. This could be a defect in the liberation of AA from membrane phospholipids, or possibly reduced incorporation of AA in these stores. The observation that increasing linoleic acid, the precursor of AA, in the diet reduces blood pressure is in agreement with the hypothesis that AA in membrane phospholipids may be reduced in the hypertensive rat. In agreement with this hypothesis is the observation that daily s.c. injections of AA significantly retarded the development of hypertension in SHR, but did not alter the normal age-related increase in blood pressure of normotensive WKY rats (Bayorh, Zukowska-Grojec, Ezra, Feuerstein and Kopin, 1983). There is very little information in the literature on the fatty acid content of blood vessels, although it has been shown that supplementation of cultured bovine and human endothelial cells with linoleic acid actually reduced the AA content of phospholipids, leading to a decrease in PGI_2 production (Kaduce, Spector and Bar, 1982; Spector, Hoak, Fry, Stoll, Tanke and Kaduce, 1981). These findings suggest that endothelial cells are not able to convert linoleic acid to AA and that the mechanism of the protective effect of a linoleic acid diet on the development of hypertension requires further study. It remains to be established whether the AA content of endothelial cell and smooth muscle cell phospholipids is deficient in hypertensive rats compared

to normotensive rats, and if this is the case, whether the deficiency is due to a fault in the uptake mechanism for AA.

The majority of studies regarding hypertension and vascular PG production have been carried out on the 2-series of PGs. An alternative suggestion for the involvement of PGs in the aetiology of hypertension is suggested by the work of Tan and Mutrow (1976). These authors found an impairment in the renal production of PGE_1 in SHR; PGE_1 synthesis increased spontaneously in the control WKY strain at about 4 months of age but there was no such increase in the kidney of SHR. In the isolated mesenteric vascular bed of SHR, low concentrations of zinc chloride (Zn) potentiated pressor responses to NA in SHR and normotensive WKY, but at higher concentrations Zn inhibited responses to NA in WKY, but not in SHR. (Mtabaji, Kihara and Yamori, 1985). Since the effects of Zn may be mediated via release of dihomo-gamma-linolenic acid (DHLA; the precursor of the 1- series PGs) and PGE_1 has biphasic effects on vascular reactivity, enhancing at low doses and depressing vascular reactivity at higher doses, it is possible that Zn is acting through the release of DHLA and its subsequent conversion to PGE_1 (physiological concentrations of PGE_1 are generally vasodepressive).

Significant quantities of dihomo-gamma-linolenic acid have been found in cultured human endothelial cells

(Rastogi and Nordoy, 1980; Spector, Hoak, Fry, Denning, Stoll and Smith, 1980; Marcus, Broekman, Weksler, Jaffe, Safier, Ullman, Islam and Tack-Goldman, 1981) so theoretically PGE_1 can be synthesised by these cells. It has been shown that basal levels of human platelet PGE_1 and PGE_2 are very low but that the amount of PGE_1 is about 4 xs higher than PGE_2 (Lagarde, Guichardant and Dechavanne, 1981). This suggests that although it is generally accepted that the 2-series PGs predominate over the 1-series PGs due to the much greater levels of AA in membrane phospholipids compared to DHLA, it is possible that under normal conditions PGE_1 is released tonically by both blood vessel wall and platelets to maintain vascular tone and normal blood flow, and it is only when a stimulus such as injury is present that the more potent anti-aggregatory PG, PGI_2 is released. If this is the case, more studies are required to establish if there is a more generalised reduction in PGE_1 production throughout the vasculature of hypertensive rats, not just in the kidney, and also if possible to determine the plasma and urinary concentrations of PGE_1 in essentially hypertensive patients. Indeed, it was suggested a number of years ago that DHLA might have potential as an antithrombotic agent. DHLA supplementation in human volunteers produced an increase in the proportion of DHLA relative to AA in plasma and platelets, decreased plasma heparin-neutralising activity and caused an inhibition of ADP-induced aggregation (Kernoff, Willis, Stone, Davies and McNicol, 1977). Unfortunately work in this area has largely ceased since the advent of PGI_2, and PGE_1's

contribution to vascular homeostasis may have been underrated.

7.3 Vascular PG production in aged rats.

Consistent measurements of systolic blood pressure were obtained from old male rats and it was established that the blood pressures of these old rats were significantly greater than that of young male rats. Previous studies have used either genetically hypertensive rats or an experimental model of hypertension to investigate the possible role of vascular PGs in the aetiology of hypertension. This study has used a normal strain of rats which, because of their advanced age, had a significantly raised blood pressure compared to their young counterparts. The old rats therefore, provided a model for studying the effects of a naturally elevated blood pressure on vascular PG production.

The most striking difference in PG production by the aorta of old compared to young male rats was the marked increase in the production of all 3 PGs by homogenates of smooth muscle from old male rats. The increase in 6-keto-$PGF_{1\alpha}$ production was 6-fold, the increase in PGE_2 production was 3-fold, but the greatest increase was in $PGF_{2\alpha}$ production which was 8-fold. The profile of PG production by the endothelium of old rats was also considerably different to that of young male rats. There was a change in the 6-keto-$PGF_{1\alpha}$: $PGF_{2\alpha}$ ratio from 3.9 : 1.0 in the endothelium of young rats to 1.3 : 1.0 in the

endothelium of old male rats. PGE_2 production was not different between the two groups. Thus, the profile of PG production by smooth muscle and endothelium from old rats was changed in favour of $PGF_{2\alpha}$. In contrast, old female rats although showing an increased production of 6-keto-$PGF_{1\alpha}$ and PGE_2, showed no difference in $PGF_{2\alpha}$ production by homogenates of smooth muscle compared to young females. The endothelial cell layer from old females produced more PGE_2 and less $PGF_{2\alpha}$ than the endothelium from young females, whereas 6-keto-$PGF_{1\alpha}$ production did not differ between the two groups.

However, the basal output of 6-keto-$PGF_{1\alpha}$ from the isolated, perfused aorta did not change significantly with the increase in age in male or female rats, and in fact tended to be higher in the aged rats. The output of $PGF_{2\alpha}$, but not PGE_2, from the perfused aorta was significantly higher in old male and female, compared to young male and female rats. Similar to the situation in the young rats, old female rats released lower amounts of 6-keto-$PGF_{1\alpha}$ compared to old male rats. Paradoxically, despite there being no difference in the production of $PGF_{2\alpha}$ by homogenates of the smooth muscle and endothelial cell layers, there was a significant increase in the release of $PGF_{2\alpha}$ from the isolated, perfused aorta of old compared to young female rats.

7.3.1 Effects of NA and AII on PG release from the mesenteric bed of aged rats.

The basal output of all 3 PGs tended to be higher from the mesenteric arterial bed of old male and female compared to young male and female rats and this was significant for 6-keto-$PGF_{1\alpha}$ and PGE_2 in old male rats. The outputs of all 3 PGs also tended to be higher in old males than old females and this was significant, for 6-keto-$PGF_{1\alpha}$, at the start of the perfusion experiment.

The lower dose of NA (0.1 µg) and AII (0.1 µg) significantly increased 6-keto-$PGF_{1\alpha}$ output from the mesenteric arterial bed ofold male rats, but not of old female rats. This was similar to the situation in young male and female rats. However, the higher doses of NA and AII (1.0 µg) increased 6-keto-$PGF_{1\alpha}$ release from both old male and old female rats. NA and AII, at either dose, had no significant effect on PGE_2 or $PGF_{2\alpha}$ output from the perfused mesenteric bed of old male and female rats. NA and AII both produced an increase in the perfusion pressure of the mesenteric bed, with NA producing the greater increases. The NA-induced changes in perfusion pressure (expressed as a percentage) were significantly greater, at both doses, in old compared to young male rats. There was no significant difference in the pressor response to AII when comparing young and old female rats.

There is very little information in the literature on PG formation by the vasculature of aged animals. Two previous studies, one using normotensive rats and the

other one using SHR have shown that PGI_2 generation by homogenates of aorta and by intact aorta increases up to 20 weeks of age (Panganamala et al., 1981; Pace-Asciak and Carrara, 1979). In a study on genetically obese rats, PGI_2 release from aortic rings was greater in rats of 14-16 months compared to rats of 6-8 months of age (Landgrof-Leurs, Loy, Christea, Weber, Seiss, Herberg and Landgrof, 1981). However, another study showed that there was no difference in the release of PGI_2 from the aorta of 15 month and 3 month old male rats (Suzuki, Kobayashi, Hayawaka and Wado, 1985). In vitro, Chang et al., (1980) found that cultured smooth muscle cells from old rats produced less PGI_2 than those from young animals. However, a later study by the same authors, Chang et al., (1983), showed that PGI_2 release from aortic rings of mature (12 months) rats is increased compared to young (2 month) rats, but is significantly decreased in senescent (24 months) rats compared to young rats. So the rats used in the present study are comparable to the mature rats used in the study of Chang et al., (1983) and show a similar trend in producing greater amounts of 6-keto-$PGF_{1\alpha}$ from the aorta than young male rats. It is not possible to comment on the increased production of $PGF_{2\alpha}$ by the aorta of old male rats in the present study as this PG was not measured by the previous authors. Thus, the evidence to date suggests that PGI_2 production by the aorta of the rat increases from an early age until it reaches a peak at about 'middle-age' whence it declines towards senescence. The mechanism of this bell-shaped curve of vascular PGI_2 generation with age

remains to be established. It is possible that the amount of PGI_2 released from a blood vessel is a function of the mechanical effect of an increase in blood pressure with increasing age.

7.3.2 Possible effects of a reduction in anti-oxidant state with age.

Lipid peroxidation induced by free radical formation is known to occur in vitamin E deficiency, in the ageing process and possibly also the hyperlipidaemia of atherosclerosis (Slater, 1972). It is well known that lipid peroxides are perhaps the most selective and effective inhibitors of PGI_2 formation by blood vessel microsomes or by fresh vascular tissue (Bunting et al., 1976; Salmon et al., 1978). It is possible therefore, that if there is a potent inhibitory effect of lipid peroxides on PGI_2 synthetase, the endoperoxide PGH_2 might be redirected into the pathways for $PGF_{2\alpha}$ and PGE_2 synthesis. The accumulation of lipids in atherosclerotic plaques could contribute to the formation of lipid peroxides. Furthermore it has been shown that there is a positive correlation between the apparent increased levels of lipid peroxides and the severity of atherosclerotic lesions (Glavind, Hartmann, Clemmesen, Jessen and Dam, 1952). High serum levels of low-density lipoproteins (LDL) contribute to the process of endothelial injury and they may also be mitogenic and contribute to the formation of the atherosclerotic plaque. Moreover, LDL has been found to inhibit PGI_2 synthesis by human endothelial cells in culture (Nordoy,

Svensson, Wiebe and Hoak, 1978). Conversely, high-density lipoproteins (HDL) have been correlated in epidemiological studies with a decreased incidence and progression of atherosclerotic disease and this has been suggested to be due to their ability to transport cholesterol away from aortic smooth muscle cells in culture, indicating their possible action *in vivo*. A negative correlation was found between the activity of the PGI_2 synthetase and the amount of LDL cholesterol; the reverse was true for HDL cholesterol (Beitz and Forster, 1981). Interestingly, these authors found that LDL cholesterol had less of an inhibitory effect, and HDL cholesterol a greater stimulatory effect on porcine aorta PGI_2 synthetase, from female compared to male subjects (Beitz and Forster, 1981). These results suggest that the difference in the risk of cardiovascular disease and thrombosis between men and women despite similar plasma concentrations of lipoproteins (Gordon, Castelli, Hjortland, Kannel and Dawber, 1977) may be related to a differential effect of the lipoproteins isolated from women and men on blood vessel PGI_2 synthetase.

Further support for the involvement of lipid peroxides in PG metabolism comes from the results of studies on vitamin E and selenium, two components of the antioxidant potential of plasma and tissues. Vitamin E inhibits lipid peroxidation, whereas selenium acts indirectly as an antioxidant through its incorporation into the enzyme, selenium-dependent glutathione peroxidase (GSH-peroxidase). This enzyme catalyses the reduction of

lipid hydroperoxides formed from PUFAs and thus has an important role in the detoxification and protection of cell membranes against oxidative damage. The formation of PGI_2 by rat or rabbit aorta has been shown to decrease when vitamin E is low or absent from the diet (Chan and Leith, 1981; Karpen, Merola, Trewyn, Cornwell and Panganamala, 1981; Chan, Pritchard and Choy, 1983). One could speculate that a decrease in vitamin E levels with age could be responsible for the decrease in blood vessel PGI_2 production via increased production of lipid hydroperoxides exerting an inhibitory effect on PGI_2 synthetase. Unfortunately, because of the large overlap in the values of human vitamin E levels measured in different age groups by different workers, it is not possible to say whether vitamin E levels decrease with age or not (for review see Farrell, 1980).

7.4 Human vascular PG production.

The patients used in this study of human vascular PG production were taking a large and varied range of medications making the analysis of the effects on PG production rather complex. However, the use of the different drugs was distributed randomly amongst the age groups and amongst smokers, ex-smokers and non-smokers, so these parameters should not have affected the results regarding drug treatment and vice versa. The effects of different drug treatments had no effect on the production of $PGF_{2\alpha}$ or PGE_2 by homogenates of aorta or saphenous vein, and had rather varied and inconsistent effects on

6-keto-$PGF_{1\alpha}$ production by homogenates of male and female aorta and saphenous vein.

7.4.1 Male and female differences in vascular PG production

The profile of PG production by blood vessels of female subjects was slightly different from that of the males. As was observed for males, the aorta from females produced similar amounts of the three PGs. The profile of PG production by the saphenous vein of females was more complex with differences being found in different age groups. In the 41-50 age group, $PGF_{2\alpha}$ was the major PG produced with lesser amounts of 6-keto-$PGF_{1\alpha}$ and PGE_2; in the 51-60 age group, 6-keto-$PGF_{1\alpha}$ was the major PG produced with lesser amounts of $PGF_{2\alpha}$ and PGE_2; and in the 61-70 age group, $PGF_{2\alpha}$ was the major PG produced with lesser amounts of 6-keto-$PGF_{1\alpha}$ and PGE_2. These differences in the profile of PG production reflected significant differences in 6-keto-$PGF_{1\alpha}$ production with age; the veins of women aged 51-60 produced significantly more ($P<0.01$) 6-keto-$PGF_{1\alpha}$ and significantly less ($P<0.01$) $PGF_{2\alpha}$ compared to the veins of women aged 41-50 or 61-70 years of age. There was a change in the 6-keto-$PGF_{1\alpha}$ to $PGF_{2\alpha}$ ratio from 2.07 : 1.0 in the 51-60 age group to 0.34 : 1.0 and 0.45 : 1.0 in the 41-50 and 61-70 age groups respectively. PGE_2 production did not vary significantly with age. There were no significant differences in the production of the 3 PGs by homogenates of aorta with age.

Comparing males and females in the same age group there was no significant sex difference in the aortic production of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ or PGE_2. There were significant differences however, in the production of 6-keto-$PGF_{1\alpha}$, with females in the 41-50 age group producing smaller ($P<0.01$) amounts of 6-keto-$PGF_{1\alpha}$ from vein homogenates than males in the same age group. But, females in the 41-50 and 61-70 age groups produced significantly ($P<0.01$) more $PGF_{2\alpha}$ from vein homogenates than males in the same age groups. There was no significant difference in the production of PGE_2 by veins of males compared to females.

7.4.2 Effects of age on vascular PG production.

There were no significant differences in PG production by homogenates of aorta and vein when comparing the age groups although there did appear to be a trend towards increased production of 6-keto-$PGF_{1\alpha}$ by aorta and vein with increased age. There was also a trend towards decreased production of both $PGF_{2\alpha}$ and PGE_2 by the aorta, but not by the vein with increasing age.

6-Keto-$PGF_{1\alpha}$ was the major PG released in all age groups of male subjects followed by lesser and approximately equal amounts of $PGF_{2\alpha}$ and PGE_2. There was a decline in the release of all 3 PGs in the 61-70 compared to the 41-50 years age group; this was not significant for 6-keto-$PGF_{1\alpha}$ or $PGF_{2\alpha}$ but was significant ($P<0.05$) for PGE_2. 6-Keto-$PGF_{1\alpha}$ was also the major PG released by

saphenous vein of females with lesser and similar amounts of $PGF_{2\alpha}$ and PGE_2. Because of the small number of female subjects it was not possible to determine if there were any age differences in PG production. The only sex difference apparent in the release of PGs was in the 61-70 years age group where the vein from females released significantly ($P<0.05$) greater amounts of $PGF_{2\alpha}$ than the males.

The results of the present study do not show any significant differences in the production of 6-keto-$PGF_{1\alpha}$ by aorta or vein of male subjects with age which is in agreement with the findings of Ylikorkala et al. (1982) where plasma 6-keto-$PGF_{1\alpha}$ levels did not change over the same age range as studied here. Because there were only 2 males in the 71-80 age group in this study it is not possible to say if the reduced production of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ by aorta and vein was a definite trend. There was a significant ($P<0.05$) decrease in the release of PGE_2 from venous rings of males aged 61-70 compared to males of 41-50 years. Because there were only 2 males and 2 females aged 70 years or more it was not possible to comment on these results with reference to the findings of Ylikorkala et al. (1982), where plasma 6-keto-$PGF_{1\alpha}$ levels were higher in females over 70 years compared to males of the same age. However, the plasma levels of 6-keto-$PGF_{1\alpha}$ measured in the former study by RIA (80-120 pg/ml) are considerably higher than the values obtained using GC-MS and the validity of the results obtained by RIA could be questioned.

It must be remembered in considering the results of these experiments that these patients have both essential hypertension and coronary heart disease and are not a normal population. Mehta, Mehta and Horalek, (1983) found that patients with coronary artery disease showed abnormally high plasma levels of TXB_2 and considerably lower levels of 6-keto-$PGF_{1\alpha}$ in response to exercise compared to healthy controls, although resting plasma TXB_2 and 6-keto-$PGF_{1\alpha}$ levels were comparable in the two groups of subjects. Using RIA, Uehara, Ishii, Ikeda, Atarashi, Takeda and Murao, (1983) found that essentially hypertensive patients had significantly lower plasma 6-keto-$PGF_{1\alpha}$ levels than controls and that there was a significant negative correlation between 6-keto-$PGF_{1\alpha}$ levels and systolic, diastolic and mean blood pressures in both hypertensive and control subjects.

These results suggest that PGI_2 may be important in the control of blood pressure in humans and that a deficiency both in basal levels and in the response to NA (Ishii et al., 1982) could be important in the pathogenesis of hypertension in humans. The findings of the present study are largely in agreement with those of Hanley and May (1985); segments of saphenous vein from patients undergoing surgery for varicose veins did not show any significant difference in the basal output of PGI_2 with respect to age, sex or smoking habits. It is unfortunate that the saphenous vein is the blood vessel most commonly available to researchers for the study of human vascular PG production as it is atypical in its response to PGI_2,

which causes vasoconstriction rather than vasodilation, as it does in the majority of blood vessels studied (Levy, 1978). This vessel therefore, being atypical in its response to PGI_2, may not be typical of the rest of the human vasculature in its ability to synthesise PGs.

7.4.3 Effects of smoking on vascular PG production.

This study has shown that homogenates of saphenous vein from smokers and ex-smokers produced 20% less 6-keto-$PGF_{1\alpha}$ than those from non-smokers, although this reduction was significant only for ex-smokers. There was also a significant reduction in the production of $PGF_{2\alpha}$ by homogenates of saphenous vein from ex-smokers and a significant reduction in the production of PGE_2 by smokers compared to non-smokers. Rings of saphenous vein from smokers or ex-smokers also released smaller amounts of the 3 PGs than non-smokers but this was significant only for $PGF_{2\alpha}$. Interestingly, reduced PG production by saphenous vein was not seen in female patients, although PG release from venous rings did appear to be greater in non-smokers. It is not possible to say if this is a genuine difference in the response of male and female vascular tissue to cigarette smoke because the number of female subjects was rather small. Thus, overall, smoking appears to cause an inhibition in the capacity of the saphenous vein to produce PGs and it is interesting to note that this inhibitory effect is apparent in the vessels of males who had not smoked, in the majority of cases, for over a year. More work is required to

establish the cause of this long-term inhibitory effect of cigarette smoke on blood vessel AA metabolism, and the component or components of smoke which are responsible for this potentially detrimental influence on blood vessel homeostasis.

PGI_2 production by cultured endothelial cells from umbilical veins of mothers who smoked was markedly reduced compared to endothelial cells from matched mothers who did not smoke. Interestingly, the endothelial cells from mild (< 15/day) and heavy (> 15/day) smokers were significantly less able to grow and reach confluency than cells from non-smokers (Busacca, Balconi, Pietra, Vergara-Dauden, De Gaetano and Dejana, 1984). However, cord blood platelet aggregation and thromboxane production, in response to ADP and collagen, were not significantly different in infants born to mothers who smoked compared to infants born to mothers who did not (Ahlsten, Ewald, Kindahl and Tuvemo, 1985).

Wennmalm and Alster (1983) have proposed that the inhibitory effect of cigarette smoke on PGI_2 production is due to the presence of nicotine. Nicotine, *in vitro*, dose-dependently inhibited the amount of PGI_2-like activity released from rings of rabbit aorta. In further studies involving the incubation of slices of rat aorta with [^{14}C]-AA in the presence of nicotine, it was found that nicotine competitively inhibited the formation of [^{14}C]-6-keto-$PGF_{1\alpha}$. The formation from [^{14}C]-PGH_2 was not affected, indicating that the inhibitory effect of

nicotine was on the cyclooxygenase rather than PGI_2 synthetase (Wennmalm and Alster, 1983). Extending these studies, Wennmalm (1983) established that although vascular PGI_2 formation was inhibited by nicotine, TXA_2 formation by platelets was not affected, thus pushing the system further in the pro-aggregatory direction. In an acute study, the smoking of nicotine-containing cigarettes, but not of nicotine-free cigarettes, decreased urinary 6-keto-$PGF_{1\alpha}$ in smokers but not in non-smokers (Nadler, Velasco and Horton, 1983). Other authors have failed to find an effect of nicotine on vascular PGI_2 production. In the pre-labelled ([^{14}C] AA) isolated, perfused rabbit ear nicotine failed to affect the basal release of AA or PGs, but strongly inhibited the histamine-stimulated release of PGI_2 and PGE_2 (Juan, 1981). Incubation of cultured umbilical vein endothelial cells with cigarette smoke condensate impaired basal and phorbol myristate acetate stimulated PGI_2 release, but these effects could not be attributed to nicotine or cadmium. Furthermore, the production of PGI_2 from exogenous AA was not affected by cigarette smoke condensate, suggesting that the inhibitory effects were not at the level of cylcooxygenase or PG synthetase (Reinders, Brinkman, Mourik and van de Groot, 1986). Cigarette smoke extracts also inhibited PGI_2 synthesis by human artery, rabbit and rat aorta and lung but the inhibitory effect was not due to nicotine (Jeremy, Mikhailidis and Dandona, 1985). Similarly, nicotine had no effect on the metabolism of exogenous AA or PGE_2 in isolated, perfused hamster and rat lungs (Mannisto,

Puustinen and Uotila, 1984). Another major component of cigarette smoke, carbon monoxide, had no significant effect on the metabolism or distribution of [^{14}C] AA in hamster lung (Mannisto, Puustinen and Uotila, 1984). Furthermore, neither nicotine nor pretreatment with carbon monoxide decreased PGI_2 production by rat aortic rings, and neither compound had a direct effect on ADP-induced aggregability of human PRP (Hartiala, Simberg, Uotila, 1982). In the present study, the depression in the production of all 3 PGs by saphenous vein of males who had smoked or were currently smoking compared to non-smokers does support the hypothesis that some factor from cigarette smoke, possibly nicotine, supresses cyclooxygenase activity. Thus it remains to be established which factor, or combination of factors, from the 3000 different compounds present in cigarette smoke are responsible for the effects of smoking on vascular PG synthesis.

Of interest, particularly to the advocates of the dangers of passive smoking (ie the effects of smoke from cigarette smokers on non-smokers) is the finding that in a model of passive smoking, rats inhaling sidestream smoke, there was a dose-related increase in platelet TXA_2 production and a maximum decrease in aortic PGI_2 synthesis at the lowest level of sidestream smoke (which has 3xs more carbon monoxide and nicotine than mainstream smoke). The decrease in aortic PGI_2 synthesis was more sensitive to both mainstream and sidestream smoke than the increase in platelet TXA_2 (Lubawy and Valentovic, 1985).

7.4.4 Effects of different drug treatments on vascular PG production.

Of the drugs being taken only calcium antagonists (nifedipine and verapamil) affected the production of 6-keto-$PGF_{1\alpha}$ in male patients, increasing 6-keto-$PGF_{1\alpha}$ production by homogenates of aorta but not of saphenous vein. In female patients 6-keto-$PGF_{1\alpha}$ production was increased in homogenates of aorta by β-adrenoceptor blocking agents, and increased in homogenates of saphenous vein by nitrate vasodilators and thiazide diuretics. 6-Keto-$PGF_{1\alpha}$ production by homogenates of aorta and saphenous vein was not affected by the loop diuretics (frusemide and bumetanide) and did not appear to be markedly affected by the NSAIDs although because there were only 9 patients taking these agents it was not possible to determine significance in this instance.

The observation that β-adrenoceptor drugs increased 6-keto-$PGF_{1\alpha}$ production by homogenates of female aorta agrees with the findings of Srivistava et al. (1983) who found that labetalol increased the synthesis of PGI_2 from exogenous labelled AA in rat aorta and also enhanced the synthesis of PGI_2 from endogenous AA. Brandt, Seppala, Nowak and Vapaatalo (1984) found that an i.v. dose of propranolol in human subjects increased plasma 6-keto-$PGF_{1\alpha}$ and PGE_2 but 2 other β-blockers, practolol and atenolol were without effect on plasma 6-keto-$PGF_{1\alpha}$ or PGE_2. This observation may be of relevance to this

study as a large number of the patients were taking atenolol.

Nitrates produced a similar effect to β-blockers with respect to PG production. Males taking nitrates did not show any significant difference in vascular PG production but the saphenous vein from females taking nitrates produced significantly ($P<0.05$) greater amounts of 6-keto-$PGF_{1\alpha}$ than females who were not taking this treatment. Glyceryl trinitrate (GTN) has been shown to stimulate the production of 6-keto-$PGF_{1\alpha}$ *in vitro* from cultured umbilical endothelial cells (McEvoy, Patel, Evans and Felton, 1983). At therapeutic concentrations, GTN increased the release of 6-keto-$PGF_{1\alpha}$ from human saphenous and umbilical vein after stimulation with AA (Mehta, Mehta, Roberts, Faro, Ostrowski and Brigmon, 1983). Another more recent study (De Caterina, Dorso, Tack-Goldman and Weksler, 1985) showed that the basal release of 6-keto-$PGF_{1\alpha}$ from umbilical vein endothelial cells and fragments of saphenous vein was not altered by incubation with GTN, isosorbide mononitrate and isosorbide dinitrate. However, using patients with ischaemic heart disease Rolland et al., (1984) observed that right atrial administration of isosorbide dinitrate increased aortic plasma 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ while reducing PGE_2.

7.5 Final Conclusions

This study has highlighted some differences in vascular PG production between normotensive and hypertensive rats and has shown that there are several important differences between the sexes. However, no difference was observed in PG production by the aorta and vena cava of female rats during the oestrous cycle or after short-term ovariectomy and 2 days of oestrogen and progesterone treatment. Further study is required to establish the reason for these observed differences in PG production in female rats.

The observation of greatest interest was that the vascular capacity for the synthesis of the vasoconstrictor, $PGF_{2\alpha}$, was greater in hypertensive than in normotensive rats of both sexes and this was reflected in a greater output of $PGF_{2\alpha}$ from the aorta and mesenteric bed. Regarding stimulated PG release, AII caused a significant increase in 6-keto-$PGF_{1\alpha}$ release in normotensive but not in GH rats. There was a greater pressor response to AII in the mesenteric bed from GH rats and the absence of any increase in PGI_2 release after AII may reflect a deficiency in this proposed aspect of the control of vascular tone in the GH male rat. However, the results from females do not agree with this hypothesis since AII stimulated 6-keto-$PGF_{1\alpha}$ output in female GH rats. The reason for this difference in response to AII in male and female rats requires further study.

Increase in age produced a marked stimulation in 6-keto-$PGF_{1\alpha}$ production by smooth muscle homogenates, with the greater stimulation being found in male rats. However, the basal output of 6-keto-$PGF_{1\alpha}$ from the perfused aorta did not change significantly with age, but did tend to be greater in old male and female rats. Thus the present study does not support the hypothesis that the increased incidence of vascular disorders with age is associated with a decrease in vascular PGI_2 production. Similar to the situation observed in hypertensive rats, the production of $PGF_{2\alpha}$ by homogenates, and $PGF_{2\alpha}$ output from perfused aorta and mesenteric bed were significantly greater in old compared to young rats. The responses to NA and AII in the mesenteric bed did not differ markedly between old and young rats. Whether the increase in blood pressure with age, and in the genetically hypertensive rat, is connected with increased vascular $PGF_{2\alpha}$ production merits further investigation.

In the human study, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 production by homogenates of aorta and saphenous vein were largely unaffected by the age or sex of the subjects. 6-Keto-$PGF_{1\alpha}$ production by homogenates of saphenous vein was lower in male, but not female, smokers and ex-smokers, although this was significant only for ex-smokers. However, the basal outputs of PGs, particularly 6-keto-$PGF_{1\alpha}$ from the saphenous vein were not affected markedly by age, sex or smoking habits.

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